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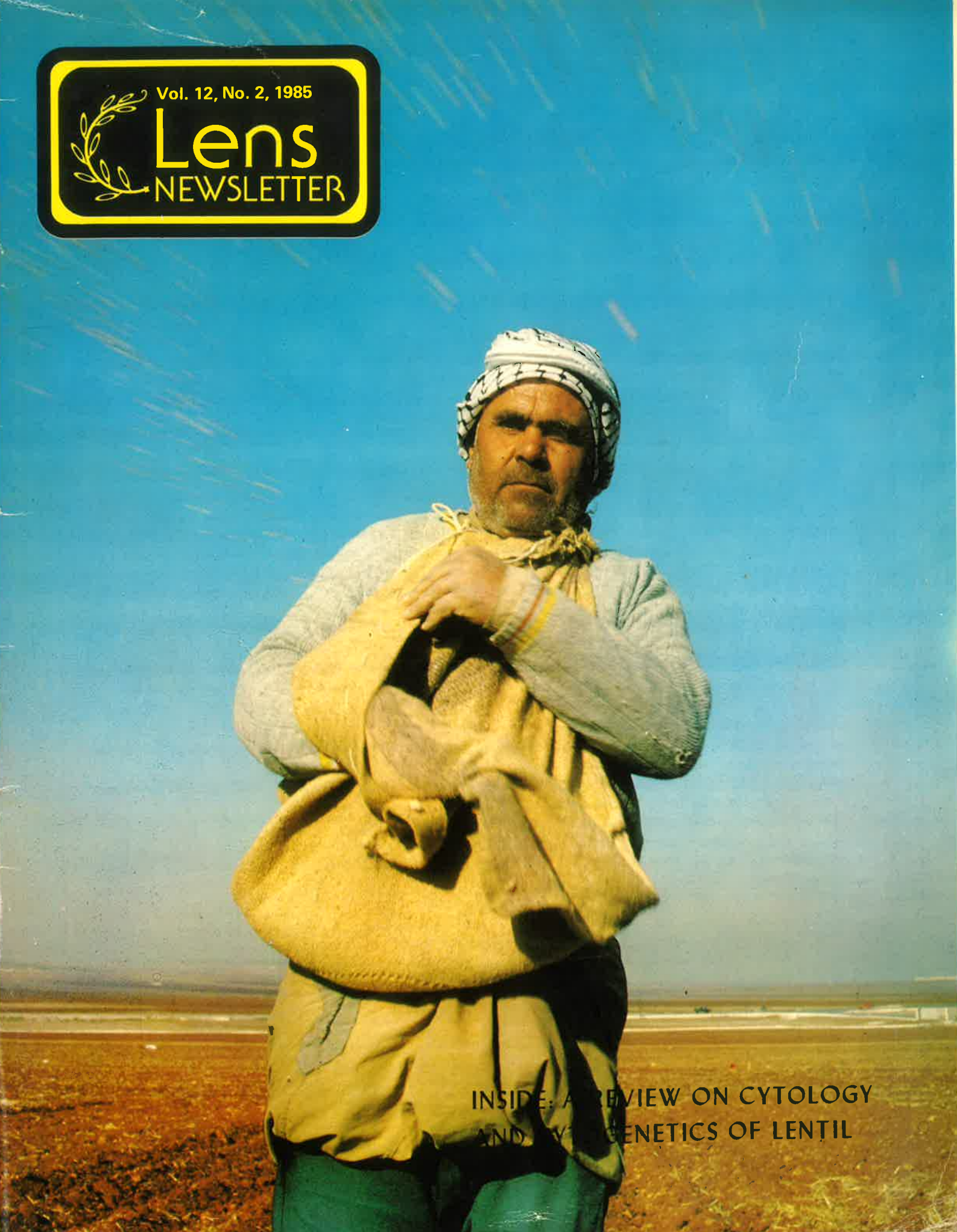
Lens NEWSLETTER



INSIDE: A REVIEW ON CYTOLOGY
AND GENETICS OF LENTIL

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LENS

LENS Newsletter is produced twice a year at ICARDA in cooperation with the University of Saskatchewan, Canada, with the financial support of the International Development Research Centre (IDRC), Ottawa, Canada. LENS, the newsletter of the Lentil Experimental News Service, is a forum for communicating lentil research results. Short research articles provide rapid information exchange, and comprehensive reviews are invited regularly on specific areas of lentil research. The newsletter also includes book reviews, key abstracts on lentils, and recent lentil references. The Lentil Experimental News Service provides information on lentil research free of charge through a question and answer service, photocopies, and searches of a lentil document collection.

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COVER PHOTO: A Syrian farmer broadcasts lentil seeds in the traditional way.

Photo credit: Murtada Seraj-El-dine, ICARDA.

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Lens NEWSLETTER

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REVIEW ARTICLE

Cytology and cytogenetics of lentils

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Abstract

Karyotypes of all lentil species consist of seven pairs of chromosomes with median or near-median centromeres. A secondary constriction (often faint) occurs near the centromere of one or, rarely, two pairs of chromosomes. Crossing relations and chromosome pairing in the F_1 revealed two "crossability" groups or species: *Lens culinaris* and *L. nigricans*. Both species were subdivided into subspecies as follows: *L. culinaris* spp. *culinaris*, *L. culinaris* spp. *orientalis*, *L. culinaris* spp. *odemensis*; and *L. nigricans* ssp. *nigricans*, and *L. nigricans* ssp. *ervoides*. F_1 hybrids within each species had various degrees of fertility. Crosses between *L. culinaris* and *L. nigricans* can be made using embryo culture. The genus *Lens* can thus be considered as one large gene pool from the plant breeding standpoint, but as two isolated gene pools from the evolutionary standpoint.

Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest domesticated crops, but detailed genetic, cytologic, and cytogenetic studies have been made of this genus only in the last 30 years. Even today, only limited information is available on *Lens*. Muehlbauer and Slinkard (1981) reviewed the current status of lentil genetics. The present review updates the information on the cytology and cytogenetics of the various lentil species, and the crossing relations between them, to familiarize lentil breeders with the extent of the lentil germplasm, or gene pool, available.

Variation in karyotypes

The first karyotype studies on lentil were reported by Bhattacharjee (1951, 1953), Sen and Ghosh (1955), and Sharma and Mukhopadhyay (1963). They confirmed earlier reports of $2n = 14$. Subsequently, a series of reports were published on refinements of the root tip technique for karyotype studies (Shahla *et al.* 1976; Sarbhoy 1980; Tiwari and Tiwari 1983). These early studies were followed by other more detailed studies.

A. L. culinaris Medik. (Syn: L. esculenta Moench)

The different karyotypes reported for *L. culinaris* are presented in Table 1. These karyotypes are quite variable, ranging from 0 to 4 metacentric (M), 0 to 7 submetacentric (SM), and 0 to 4 subtelocentric/acrocentric (ST/AO) chromosomes with 0-3 chromosomes having satellites.

In most instances, the basis for classifying the various chromosome types was not stated and varied in different studies. Accordingly, all studies in which arm ratios were given for individual chromosomes were reclassified according to the scheme of Levan *et al.* (1964) (see footnote on Table 2). Reclassification reduced variability somewhat (Table 2), in that few lines had subtelocentric chromosomes (arm ratios of 3.0-7.0) and none had acrocentric chromosomes (arm ratios greater than 7.0). These results indicate that *L. culinaris* had a primitive karyotype with mostly symmetrical chromosomes.

The number of chromosomes with a secondary constriction and a satellited chromosome ranged from 0 to 3 (Table 1). The secondary constriction in *Lens* species is usually near the centromere and difficult to detect because it is not visible in every cell, and is only visible in one homologue in some cells (Sindhu *et al.* 1983c). Consequently, some studies reporting no satellites may have failed to identify some of these satellited chromosomes. For example, Gupta and Singh (1981a) noted a characteristic centromere with a darkly-stained region flanked on either side by lighter-staining regions in Pant L-639, but they failed to identify this as a secondary constriction with a satellite. Subsequently, Sharma and Gupta (1982) reported that Pant L-639 had a satellited chromosome.

Naithani and Sarbhoy (1973) reported that *L. culinaris* had two chromosomes with secondary constrictions near the centromere (chromosomes nos. 2 and 3), which was also reported by others. However,

the authors also observed two chromosomes (nos. 6 and 7) with a short terminal satellite, the only report of a short terminal satellite in any *Lens* species. If correct, then these lines had four satellited chromosomes; however, it is more likely that these latter two chromosomes with the short terminal satellite were subtelocentric chromosomes (refer to Figure 13; Naithani and Sarbhoy 1973).

B. L. orientalis (Boiss.) Handle-Mazetti

Only five karyotype studies have been reported on *L. orientalis* (Table 1). Most of them indicate the presence of one satellited chromosome and up to three subtelocentric/acrocentric chromosomes. However, when the results of Sindhu *et al.* (1984) were reclassified according to the Levan *et al.* (1964)

Table 1. Karyotypes reported for *Lens* species.

Species	Karyotype†				Reference
	M	SM	ST/A	Number with satellites	
Lens culinaris					
ssp. *microsperma*	1	6	0	2	Bhattacharjee (1951)
6 lines	3	4	0	1	Sharma and Mukhopadhyay (1963)
8 lines	4	3	0	1	
5 lines	3	4	0	1	
4 lines	2	5	0	1	
3 lines	4	3	0	0	Sinha and Acharia (1972)
3 lines	3	4	0	0	
ssp. *microsperma*	3	2	2	2	Naithani and Sarbhoy (1973)
ssp. *macrosperma*	3	2	2	2	
-	3	0	4	0	Williams *et al.* (1974)
60L 11	1	3	3	0	Eser (1976)
ssp. *microsperma*	0	6	1	2	Prasad and Jana (1976)
No. 2, 7, and 13	2	2	3	1	Ladizinsky (1979a)
Pant. L 639	4	3	0	?	Gupta and Singh (1981b)
Pant. L 639	2	2	3	1	Sharma and Gupta (1982)
4 lines	3	2	2	0	
4 lines	3	1	3	0	
2 lines	2	4	1	0	
2 lines	2	2	3	0	Sinha and Singh (1982b)
2 lines	1	5	1	0	
1 line	4	2	1	0	
1 line	1	4	2	0	
1 line	1	2	4	0	
1 line	2	1	4	0	
1 line	1	3	3	0	
1 line	2	3	2	0	
No. 13	0	4	3	1	Buruchin and Ladizinsky (1983)
I 3847	0	7	0	1	Lavania and Lavania (1983)
-	1	3	3	1	Sindhu *et al.* (1983a).

continued

5 spp. *microsperma*	-	-	-	1	
3 spp. *macrosperma*	-	-	-	1	
1 spp. *macrosperma*	-	-	-	2	Sinha and Keswani (1983)
1 spp. *microsperma*	-	-	-	3	
Lens orientalis					
-	3	4	0	0	Williams *et al.* (1974)
No. 22,23,24,26	2	2	3	1	Ladizinsky (1979a)
No. 24	0	4	3	1	Buruchin and Ladizinsky (1983)
-	1	3	3	1	Sindhu *et al.* (1983a)
11 lines	1	3	3	1	Sindhu *et al.* (1984)
Lens nigricans					
-	4	0	3	1	Sinha and Acharia (1974)
-	4	2	1	0	Williams *et al.* (1974)
Turkish line	4	0	3	1	Ladizinsky (1979a)
-	1	3	3	1	Sindhu *et al.* (1983a)
Lens ervoides					
-	0	4	3	1	Sindhu *et al.* (1983a)
5 lines	0	4	3	1	Sindhu *et al.* (1983b)
Lens montbretii = *Vicia montbretti*					
Turkey and Iraq	0	2	4	1	Ladizinsky and Sakar (1982)

† M,SM,and ST/A refer to metacentric, submetacentric, and subtelocentric/acrocentric chromosomes, respectively.

scheme, there were no subtelocentric/acrocentric chromosomes (Table 2), indicating again the primitive nature of the *Lens* karyotype.

C. L. nigricans (Bieb.) Godr.

Only four karyotype studies have been reported on *L. nigricans* (Table 1). Most of them indicate the presence of a satellited chromosome and up to three subtelocentric/acrocentric chromosomes (Table 1). However, when the results of Sinha and Acharia (1974) were reclassified according to the Levan *et al.* (1964) scheme, there was still one subtelocentric chromosome (Table 2). Since they were analyzing a translocation heterozygote, it is possible that this subtelocentric chromosome was involved in the translocation, having exchanged a long arm for a short terminal segment from a non-homologous chromosome.

Sinha and Acharia (1974) studied cytology of a line of *L. nigricans* from Czechoslovakia. They noted many cytological abnormalities, such as multivalents, suggesting the line was a translocation heterozygote; they also suggested that *L. nigricans* was not stable. However, only one line was involved and other studies have shown that *L. nigricans* is cytologically normal. There are probably unstable lines in other *Lens* species as well.

D. L. ervoides (Brign.)

Only one karyotype study (Sindhu *et al.* 1983c) has been reported on *L. ervoides*; it indicated the presence of a satellited chromosome and three acrocentric chromosomes (Table 1). However, when the arm length data were reclassified according to the Levan *et al.* (1964) scheme, there were no acrocentric or subtelocentric chromosomes (Table 2).

Table 2. *Lens* karyotypes reclassified, according to the Levan[+] scheme, for studies in which arm ratios were reported.

Species	Karyotype**				Reference
	M	SM	ST/A	Number with satellites	
Lens culinaris					
4 lines	3	4	0	0	
3 lines	5	2	0	1	
2 lines	5	1	1	1	Sinha and Acharia (1972)
1 line	6	1	0	1	
1 line	4	3	0	1	
1 line	4	3	0	0	
1 line	3	4	0	1	
1 line	2	4	1	1	
1 line	0	7	0	0	
60 L 11	4	2	1	1	Eser (1976)
ssp. *microsperma*	3	4	0	2	Prasad and Jana (1976)
Pant. L 639	4	3	0	?	Gupta and Singh (1981b)
5 lines	4	3	0	0	
3 lines	3	4	0	0	
2 lines	6	0	1	0	
2 lines	5	2	0	0	Sinha and Singh (1982b)
2 lines	4	2	1	0	
2 lines	3	3	1	0	
1 line	6	1	0	0	
1 line	5	1	1	0	
1 line	4	1	2	0	
1 line	3	2	2	0	
Lens orientalis					
11 lines	4	3	0	1	Sindhu *et al.* (1984)
Lens nigricans					
	3	3	1	1	Sinha and Acharia (1974)
Lens ervoides					
5 lines	4	3	0	1	Sindhu *et al.* (1983b)

[+]According to Levan et al. (1964), metacentric chromosomes have arm ratios of 1.0 to 1.7, submetacentric chromosomes have arm ratios of 1.7 to 3.0, subtelocentric chromosomes have arm ratios of 3.0 to 7.0, and acrocentric chromosomes have arm ratios >7.0.

**M, SM, and ST/A refer to metacentric, submetacentric, and subtelocentric/acrocentric chromosomes, respectively.

E. L. montbretii (Fisch. and Mey.)

Ladizinsky and Sakar (1982), the only researchers to study the karyotype of *L. montbretii*, reported 2n = 12 in contrast to 2n = 14 reported for all other *Lens* species. In addition, *L. montbretii* has one acrocentric and three telocentric chromosomes. The satellited chromosome has the secondary constriction near the end of the chromosome, in contrast to the near median position of the secondary constriction in other *Lens* species. These differences, plus gross morphological differences, prompted Ladizinsky and Sakar (1982) to place this taxon in the genus *Vicia* as *V. montbretii* (Fisch. and Mey). This study should end further speculation about the relationship of this taxon to *Lens*.

F. Summary of karyotype studies

Karyotype studies justify the return of *L. montbretii* to the genus *Vicia* as *V. montbretii*. However, there is little basis to distinguish among the karyotypes of *L. culinaris*, *L. orientalis*, *L. nigricans*, and *L. ervoides*. All four species have seven metacentric or submetacentric chromosomes, often with one or, occasionally, two secondary constrictions near the centromere. These results confirm that the genus *Lens* has a primitive, generalized and homogeneous karyotype. It may be concluded that karyotype modifications played a minor role in the evolution of the genus *Lens*.

Further differentiation of the karyotype is possible using various banding techniques, such as Giemsa C-banding (Lavania and Lavania 1982). Karyotype preparation has now been facilitated by the development of a computer program for chromosome measurement (McGurk and Rivlin 1983). Faster, more refined methods for karyotype studies may result in further differentiation among the karyotypes of *Lens* species.

Meiotic, cytogenetic, and electrophoretic studies in lentil

A. Meiosis in normal lentil

Lentil buds are extremely small when they are undergoing meiosis and are difficult to study. Bhattacharjee (1953), the first to study meiosis in *L. culinaris*, noted two bivalents attached to the nucleolus, suggesting two satellited chromosomes corresponding to the two secondary constrictions reported in the earlier study of mitosis in *L. culinaris* (Bhattacharjee 1951). Ahmad (1977) reported one to four bivalents attached to the nucleolus, but most commonly only one. Sinha and Acharia (1975) reported variability among *L. culinaris* cultivars for the number of ring vs rod bivalents. Sinha and Keswani (1983) concluded that variation in chromosome morphology, total chromatin length, and the length of chromosomal segment between the primary and secondary constrictions may account for the genetic differences between *L. culinaris macrosperma* and *L. culinaris microsperma* as distinguished by Barulina (1930).

A series of studies on *L. culinaris* following irradiation treatment with or without a chemical mutagen were reported by Sinha and Godward (1969, 1972a, 1972b), Jana *et al.* (1974), Sinha (1977, 1979), Sinha and Singh (1982a), and Dixit and Dubey (1983). They reported a wide range of cytological abnormalities.

B. Meiosis in interspecific hybrids

Ladizinsky (1979a) was the first to study meiosis in interspecific hybrids among the various species of *Lens*. He noted five bivalents and one heteromorphic quadrivalent at Metaphase I of the F_1 of *L. culinaris* x *L. orientalis* and concluded that they differed by a single chromosome interchange. Buruchin and Ladizinsky (1983) studied meiosis in these F_1 plants in more detail. The similar karyotype of the two parental species and the heteromorphic quadrivalent at Metaphase I of the F_1 indicated that a submetacentric chromosome and an acrocentric chromosome were involved in the translocation. Since no karyotypic differences were noted between the two parental species, the chromosomes involved must have broken at similar distances from the centromeres. The low frequency of homomorphic ring bivalents was thought to indicate that breakage occurred in the long arm of the acrocentric chromosome, and that chromosomes of similar size in the translocation complex had homologous centromeres. They reported an overwhelming number of cells with alternate-1, relative to alternate-2, orientation of the quadrivalent, and attributed this to the similar length of the zigzag diagonals of alternate-1, which apparently induced greater stability. Pole-centromere specificity was proposed to explain the absence of adjacent-2 orientation and the orientation of homologous centromeres to opposite poles, even when they were located in different bivalents.

These F_1 plants of *L. culinaris* x *L. orientalis* were partially sterile, but still produced an abundance of F_2 seed. The F_2 populations developed normally and segregated for a series of qualitative traits (Ladizinsky 1979b).

Ladizinsky (1979a) also studied Metaphase I in the F_1 of *L. culinaris* x *L. nigricans*. He noted a

relatively large number of univalents and rod bivalents, plus a few multivalents, especially trivalents and quadrivalents, and a few chains of five, six, or seven chromosomes. The most common chromosome association was two univalents, three bivalents, and two trivalents. He observed a quadrivalent plus a pentavalent in a few cells, and a trivalent plus two quadrivalents in other cells. He interpreted these results to indicate that *L. culinaris* and *L. nigricans* differed by three interchanges. This abnormal chromosomal pairing resulted in a low level of fertility, but a fair number of F_2 seeds were still produced. Thus, gene exchange between these two species is possible.

Goshen *et al.* (1982) studied the F_1 and F_2 of *L. culinaris* x *L. nigricans*. About 20% of the flowers on the F_1 plants produced seed. Pollen fertility in 108 F_2 plants ranged from sterility as great as in the F_1 (about 10% of the F_2 plants), to fertility as high as in the parental species (about 18% of the F_2 plants). Eight fully fertile F_2 plants were backcrossed to *L. culinaris*. Two of these BC F_1 hybrids were characterized by seven bivalents, a chromosome arrangement identical to *L. culinaris*. However, the remaining six BC F_1 hybrids were characterized by five bivalents and a quadrivalent, which differed from *L. culinaris* by one translocation. Since these latter six fully fertile F_2 plants were not intercrossed, it is not known whether they differ from *L. culinaris* by the same translocation. However, they are obviously cytogenetically closer to *L. culinaris* than to *L. nigricans*, showing how rapidly introgression can take place in fertile progeny.

Ladizinsky *et al.* (1983) intercrossed a series of *L. nigricans* collections. Some were small disjunct populations from secondary and man-made habitats throughout southern Europe; others were large populations from primary habitats in Spain, Italy, and Yugoslavia; and two lines were from the Middle East. The latter two lines crossed readily with *L. culinaris* to produce an F_1 heterozygous for three translocations as noted earlier by Ladizinsky (1979a) and Goshen *et al.* (1982). No seed was produced when these Middle East sources of *L. nigricans* or *L. culinaris* were crossed with the *L. nigricans* populations from southern Europe. However, most of the latter populations were fully fertile and cytogenetically alike when intercrossed, except for one population which differed by one translocation and two other populations that differed by four translocations and a paracentric inversion. These small *L. nigricans* populations in man-made habitats in southern Europe were interpreted as escapes from cultivation. Their incompatibility with *L. culinaris* and the Middle East populations of *L. nigricans* indicates that they were probably derived from a domesticated form of *L. nigricans* (Ladizinsky *et al.* 1983). Thus, these populations of *L. nigricans* may have been domesticated independently in southern Europe and became even further isolated genetically and cytogenetically from *L. culinaris*. Only one mutation to pod-dehiscence is needed for the transition of a domesticated lentil back to a wild lentil. The probability of finding a cultivated *L. nigricans* today is very low.

Ladizinsky (1979a) also crossed *L. orientalis* x *L. nigricans* from the Middle East. Four albino F_1 plants resulted, which died. Coupled with the above results, he concluded that *L. orientalis* and *L. nigricans* from the Middle East differed from each other by at least two, and possibly four, chromosome interchanges.

C. Other cytogenetic studies

Solh and Alahaydoian (1980) treated germinating lentil seeds with colchicine and induced tetraploid root tips, but they did not carry the experiment further. Gupta and Singh (1982) produced tetraploid lentil plants by placing cotton plugs saturated with colchicine solution on the apical bud (0.5% colchicine for 10 hours). Tawar and Gour (1984) successfully produced tetraploid lentil by treating germinating seed with 0.25% colchicine. Sharma *et al.* (1983) studied autctetraploid lentil in the C_2 and noted typical gigantism of various morphological traits (Tawar 1976; Tawar and Tiwari 1981).

Malaviya and Shukla (1981) reported a spontaneous chimera in lentil which had two triploid branches, while the main shoot was hexaploid. Malaviya and Shukla (1983a) noted that only one seed was produced (on the hexaploid branch); it resulted in a diploid plant, but with extra large seeds. They also found several spontaneous tetraploids. They concluded that polyploidy would not be maintained in natural lentil populations. Malaviya and Shukla (1983b) studied cytology of the triploid-hexaploid chimera and noted many abnormalities resulting in almost complete male and female sterility (one seed set on the hexaploid branch as noted above).

Gupta *et al.* (1984) are continuing their work on tetraploid lentil with the idea of eventually developing a set of primary trisomics in lentil to assist with genetic mapping. Gupta and Singh (1981b) and Gupta *et al.* (1984) are also irradiating lentil to establish a set of translocation testers and to develop a complete heterozygous interchange stock involving all 14 chromosomes.

D. Electrophoretic studies

Ladizinsky (1979c) used seed-protein electrophoresis in a further attempt to elucidate relationships among *Lens* species. He noted similar protein profiles for *L. culinaris* and *L. orientalis*, further supporting the close genetic relationship between these two species, even though they differed by one translocation (Ladizinsky 1979a). The seed-protein profile of the one *L. nigricans* line from the Middle East was also similar, further supporting the close genetic relationship of these three species. Unfortunately the *L. nigricans* lines from southern Europe were not available at this time and were not tested for their relationship to other *Lens* species. The seed-protein profile of *L. ervoides* had 12 bands which migrated faster than the 11 bands in the other three species. A 1:1 mixture of *L. culinaris*/*L. ervoides* protein extract was then tested, and only four of the bands were equivalent in the two species. These results are consistent with morphological differences and the incompatibility of *L. culinaris* and *L. ervoides*.

Skibinski and Savage (1981) noted aspartate aminotransferase polymorphism in *L. culinaris*. A fast moving locus had a fast and a slow variant, both of which were found in over 10% of the accessions tested, based on only two plants/accession. Presumably a much higher level of polymorphism would have been detected with more plants in each accession. Skibinski *et al.* (1984) made a more complete study (10 plants/accession) and reported that 59% of 298 accessions were polymorphic for both alleles of the aspartate aminotransferase locus. They also reported significant variation in allele frequency and polymorphism among genographic areas. In addition, a high frequency of the fast allele was associated with late flowering and maturity and with low yield. The frequency of outcrossing was estimated from the observed heterozygosity to be about 1%.

Skibinski and Warren (1984) studied variation in 20 soluble enzyme loci in *L. culinaris*, *L. orientalis*, *L. nigricans* (from southern Europe), and *L. ervoides*. *L. culinaris* and *L. orientalis* had the greatest genetic similarity, but there was little similarity between the other species. A phylogenetic dendrogram was constructed from the genetic distance calculated from the data. In it, a hypothetical ancestor gave rise to both *L. culinaris* (with little differentiation) and *L. orientalis* (with much differentiation). Then, a second hypothetical ancestor (well differentiated from the first) gave rise to *L. nigricans* from southern Europe (with much differentiation) and to *L. ervoides* (with even more differentiation). Perhaps if *L. nigricans* from the Middle East had been included, the relationship between *L. ervoides* might have been much closer.

Zamir and Ladizinsky (1984) examined segregations for eight allozymes and epicotyl color in F_2 hybrids of *L. culinaris* and *L. orientalis*. They noted linkage (14 map units) between the gene for epicotyl color (Gs) and the fast migrating allele for glutamate oxaloacetate transaminase (Got-2). Linkage (25 map units) was also detected between the gene for epicotyl color (Gs) and the fast migrating allele for Malic enzyme (Me-1). A third linkage (21 map units) was detected between the third allele of glutamate oxaloacetate transaminase (Got-3) and the allele for alcohol dehydrogenase (Adh-1). Additional studies of this type are needed to better characterize the genetic variability in *Lens* and to assist lentil breeders in crop improvement efforts.

Revision of the genus Lens

Ladizinsky *et al.* (1984) made additional genetic and cytogenetic studies of the genus *Lens* and proposed a revision of the genus. They made intraspecific crosses in *L. orientalis* and noted that various sources differed by 0,1,2,3, and 4 translocations as indicated by chromosome pairing and varying levels of fertility in the F_1.

Ladizinsky *et al.* (1984) noted variability for stipule orientation in *L. nigricans*. One form had horizontal stipules (from the Eastern Mediterranean area and Turkey), while the other form had vertical stipules (W. Turkey and S. Europe). Intraspecific hybrids among various sources of *L. nigricans* with horizontal stipules had normal pairing during meiosis and were fully fertile. Intraspecific hybrids among various sources of *L. nigricans* with vertical stipules were mostly normal, but one had one translocation, while two others differed by four translocations and one paracentric inversion. These two stipule types were widely separated genetically, since most fertilized pods aborted within two weeks. Four albino F_1 plants were finally produced. One had a green sector which eventually flowered. Chromosome pairing in this F_1 indicated that the two stipule types of *L. nigricans* differed by four translocations.

Next, Ladizinsky *et al.* (1984) intercrossed the two stipule types of *L. nigricans* with representatives of the three different cytotypes of *L. orientalis*. All F_1 pods aborted in the crosses between *L. orientalis* and the vertical stipule *L.*

nigricans, whereas the crosses between *L. orientalis* and the horizontal stipule *L. nigricans* were similar to the intraspecific hybrids of *L. orientalis* (up to three translocations, but partial fertility).

Ladizinsky *et al.* (1984) intercrossed various sources of *L. ervoides* and noted normal pairing and fertility in the F_1. Crosses of *L. ervoides* with *L. orientalis* and the horizontal stipule *L. nigricans* resulted in early pod abortion. However, the cross between *L. ervoides* and the vertical stipule *L. nigricans* produced F_1 pairing, indicating that these two taxa differed by two translocations and possibly by a paracentric inversion.

Finally, Ladizinsky *et al.* (1984) crossed *L. culinaris* with the various wild species. F_1 chromosome pairing in hybrids between *L. culinaris* and various sources of *L. orientalis* indicated that they did not differ by interchange or only by one, except for several *L. orientalis* sources which differed so much that pod abortion occurred. Likewise, early pod abortion occurred in the F_1 hybrids of crosses between *L. culinaris* and *L. ervoides* and vertical stipule *L. nigricans*. However, they noted that the F_1 between *L. culinaris* and horizontal stipule *L. nigricans* differed by three translocations, but were partially fertile.

Ladizinsky *et al.* (1984) summarized these results to indicate two main "crossability" groups, with at least partial fertility in hybrids within each group and early pod abortion when hybrids were attempted between the two groups. Thus, they proposed the following subdivision of the genus *Lens* (see the key in their original report):

New	Old
L. culinaris ssp. *culinaris*	*L. culinaris*
L. culinaris ssp. *orientalis*	*L. orientalis*
L. culinaris ssp. *odemensis*	*L. nigricans* (horizontal stipule)
L. nigricans ssp. *nigricans*	*L. nigricans* (vertical stipule)
L. nigricans ssp. *ervoides*	*L. ervoides*

While these results indicate that the genus *Lens* consists of two independent germplasm pools, Cohen *et al.* (1984) used embryo culture to produce hybrids between *L. culinaris* spp. *culinaris* and *L. nigricans* spp. *ervoides*. Hybrids were also obtained between *L. culinaris* spp. *odemensis* and *L. nigricans* spp. *ervoides*. Ladizinsky and co-workers concluded that from the plant breeding standpoint, the genus *Lens* can be considered one genepool.

Discussion

Karyotypic studies of various taxa in the genus *Lens* indicate that this genus has seven pairs of chromosomes, usually with median or sub-median centromeres. Most taxa are characterized by a chromosome with a secondary constriction (often faint) near the centromere. Thus, *Lens* has a primitive, generalized, and homogeneous karyotype, and karyotype modification has played a minor role in the evolution of the genus. Zohary (1972) and Zohary and Hopf (1973) concluded that *L. culinaris* originated from *L. orientalis* in the Near East.

Study of pairing relationships within and between various taxa of *Lens* indicates a fairly high frequency of translocations. Apparently, most break points were at similar distances from the centromere, so that the true acrocentric chromosomes (arm ratios > 7.0) rarely occurred. However, these translocations plus an embryo breakdown (incompatibility) factor were used to subdivide the genus *Lens* into two crossability groups on species: *L. culinaris* and *L. nigricans*. Even so, Cohen *et al.* (1984) used embryo culture to overcome this incompatibility, providing a mechanism whereby lentil breeders can utilize all genetic variability within the genus *Lens* in their efforts to improve the cultivated lentil. This finding should be of great benefit to lentil breeders throughout the world.

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RESEARCH ARTICLES

Breeding and Genetics

Correlation and regression studies in lentil cultivars

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Abstract

Associations between the growth characters of plant height, number of branches/plant, number of pods/plant, yield/plant, and test weight were determined in 10 lentil cultivars. Grain yield was positively correlated with number of pods/plant and cultivar test weight. Multiple correlation of grain yield with plant height, number of branches, and pods/plant was highly significant. The partial regression values were also significant.

Introduction

Among the winter pulses, lentil is the most important pulse crop after chickpea in India. It is cultivated in the northern and eastern parts of the country, as well as in central India's rice belt. Improvements in lentil (*Lens culinaris* Med.) have resulted in short duration varieties such as Precoz, L-4076, and L-830. Correlation studies between different agronomic traits, such as yield components and yield, would help plant breeders and agronomists to conduct further studies on growth and yield attributes. Although lentil was subject to such study (Tikka *et al.* 1977; Chauhan and Sinha 1982; Sarwar *et al.* 1982), no special attention was paid to the association of many yield components with yield in the new lentil plant types. This study investigates this association in new lentil cultivars.

Materials and Methods

Ten cultivars of varying growth habits were planted in a randomized complete block design with three replications in the beginning of the 1983 winter season (mid-November) at the Indian Agricultural Research Institute Farm in New Delhi. The genotypes, although few in number, were randomly selected from the IARI collection and included representatives from Central and North India and also South America. Hence, they represent a wide spectrum of variation in lentils. The plots were 5.0 m x 3.0 m with an intra-row spacing of 30 cm. The crop was raised with the recommended package of practices under irrigation. Five plants/plot were randomly selected for observations on yield and other important ancillary characters such as plant height, number of branches, number of pods, and test weight. Multiple correlation and partial regressions of grain yield with four ancillary characters were worked out and tested for significance according to Snedecor's (1946) method. The multiple regression equation between grain yield and the four yield components was: $Y = a + b_{y1.234}x_1 + b_{y2.134}x_2 + b_{y3.124}x_3 + b_{y4.123}x_4$, where $b_{y1.234}$, $b_{y2.134}$, $b_{y3.124}$, and $b_{y4.123}$ are the partial coefficients.

Results and Discussion

There was a highly significant positive correlation between grain yield and other important ancillary characters: plant height, number of branches, number of pods, and test weight (Table 1). Plant height had a highly significant positive correlation with number of branches, number of pods, and test weight. The relationship between number of branches and number of pods was positive and highly significant, but negative and significant for number of branches and test weight. Number of pods was positively and significantly correlated with test weight.

The multiple correlation and partial regression values obtained for grain yield with plant height, number of branches, number of pods, and test weight are as follows:

$$R_{y(1234)} = 0.655^{**}$$
$$b_{y1.234} = -0.0279^{**}$$
$$b_{y2.134} = -0.0163$$
$$b_{y3.124} = 0.0185^{**}$$
$$b_{y4.123} = 0.0179^{**}$$

Table 1. Correlations between lentil characters.

Character	Plant height (cm)	Number of branches/ plant	Number of pods/ plant	Test weight (g)
Yield/plant (g)	0.616**	0.231**	0.685**	0.715**
Plant height (cm)		0.733**	0.835**	0.318**
Number of branches/plant			0.727**	0.180*
Number of pods/plant				0.310**

* P > 0.01 < 0.05 (degrees of freedom = 148).
**P < 0.01.

Where:

Y = grain yield/plant
Y_1 = plant height (cm)
Y_2 = number of branches/plant
Y_3 = number of pods/plant
Y_4 = test weight (g).

These values showed that the multiple correlation of grain yield with plant height, number of branches, number of pods, and test weight was highly significant. Keeping all the other components as constants, the partial regression coefficient of grain yield with plant height was negative and highly significant. The partial regression between grain yield with number of branches was negative and insignificant, keeping plant height, number of pods, and test weight constant. The partial regression coefficient of grain yield with number of pods was positive and highly significant, keeping plant height, number of branches, and test weight constant. The partial regression coefficient of grain yield with test weight was positive and highly significant, keeping plant height, number of branches, and pods constant.

The number of pods/plant and test weight increased grain yield, as was also reported by Kumar *et al.* (1983).

The contribution to increased yield of number of pods/plant and number of branches/plant, as well as the direct negative effect of plant height on yield, concur with the results of Chauhan and Sinha (1982). Thus, incorporating more pods/plant would result in high-yielding lentil cultivars.

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Pollen abortion in chemically-induced male sterile lentil

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Abstract

A comparative study of anther development and microsporogenesis in normal and chemically-induced male sterile lentil plants revealed that pollen abortion in the male steriles was associated with the abnormal development of the tapetal layer. In the male steriles induced by maleic hydrazide (MH) and

Indole butyric acid (IBA), the tapetum failed to enlarge and develop into the normal peripheral plasmodium, whereas in the coumarin-induced male steriles. the tapetal cells enlarged more than usual and were persistent. The failure of the tapetal cells to degenerate at a proper time probably deprived the developing microspores of nourishment and caused them to abort. The chemically-induced male sterile plants also had poor vasculature and some degenerated vascular tissues.

Introduction

In a study of foliar applications of some phytogametocidal compounds on lentil (*Lens culinaris* Med.) cultivars Pant-406 and JLS-2, it was observed that pollen sterility was induced by 0.1% of IBA, 0.075% of MH, and 1.0% of coumarin (Awasthi 1984; Awasthi and Dubey 1985). This paper reports the results of a comparative study on anther development and microsporogenesis in chemically-induced male sterile plants and normal fertile plants, carried out to understand the mode of pollen abortion in male sterile plants.

Materials and Methods

Flower buds, collected at various developmental stages from chemically-induced male sterile and control plants were fixed in FAA. The buds were embedded in paraffin following the usual method of dehydration with an alcohol xylol series. The material was sectioned at a thickness of 10-12 μ and stained with Haedenhai's haematoxylon (Johansen 1940).

Observations

Anther development in untreated plants

Lentil anthers are tetrasporangiate. The young anther appears as a four-lobed structure in transverse section. The hypodermal archesporial layers differentiate at the four corners of a young anther and consist of five-to-seven cells in a transverse section. The anther wall formation follows the dicotyledonous type of development as described by Davis (1966).

The mature anther wall consists of an epidermis, an endothecium, a middle layer, and a tapetum (Fig. 1). The epidermis persists in the mature anther but its cells become stretched during anther enlargement (Fig. 2). Even at maturity, greatly stretched epidermal cells can be seen adhering to the outer walls of the endothecial cells. The endothecial cells develop fibrous thickenings on their radial walls. However, the cells situated at the junction of the two pollen sacs on either side of the anther do not develop such thickenings (Fig. 3). Cells of the middle layer become stretched and degenerate during anther development. The remains of the middle layers are visible up to the formation of young microspores (Fig. 1).

The tapetum is intermediate between the secretory and plasmodial types. The entire layer of tapetum loses contact with other wall layers as the pollen mother cells enter meiosis. By the time the uninucleate pollen mother cells form in the anther, the walls of tapetal cells are dissolved and the cytoplasmic contents of these cells form a peripheral layer around the microspores (Fig. 4B). These contents do not intrude into the pollen sac, unlike in the plasmodial type of the tapetum; instead, this layer gradually disappears by the time the binucleate pollen grains form (Fig. 4A). The designation "peripheral plasmodium type" has been suggested for the behavior of such a tapetum (Shukla 1954).

Primary sporogenous cells multiply through a series of miotic divisions into many microspore mother cells (Fig. 1). Pollen mother cells grow for some time. Meiosis is generally synchronous; however, different anthers in a flower often do not develop synchronously. We occasionally observed one anther of a flower with pollen mother cells and the second anther with mature pollen grains (Fig. 4A and B). Tetrads are generally tetraheydral, but occasionally isobilateral or decussate. The microspores in a fully-formed tetrad appear to be set into a solid gelatinous matrix.

The thin walled microspores enlarge soon after separating from tetrads, and a thick smooth exine develops on their walls. The mature pollen grains are rectangular with round ends (Fig. 3), with germ pores situated at the rounded angles. The pollen grains are binucleate at the time of shedding.

At maturity, the two loculi of each anther lobe become confluent due to dissolution of the partitioning wall layers (Fig. 5). The epidermal cells of the anther wall at the junction of the two loculi remain smaller in size with a denser cytoplasm; i.e., they do not become papillate as in the other portions of the anther wall. The endothecial cells of this region do not show

characteristic thickenings. These cells seem to form a stomium at the junction of the two sporangia of a thecum, which apparently assists anther dehiscence.

Anther development in chemically-induced male sterile plants

During microsporogenesis and pollen development in the chemically-induced male sterile plants, the anther development and the initial behavior of the different wall layers followed the normal patterns of fertile plants. Later, the tapetal and endothecial layers behaved abnormally.

The MH-and IBA-induced male sterile anthers developed normally up to the stage at which pollen mother cells undergo meiosis. But in later development, pollen abortion was associated with the persistence of the tapetum. The tapetum cells failed to enlarge and develop into the normal peripheral plasmodium. In the MH-treated plants, pollen degeneration set in at the microspore tetrad stage (Fig. 6); in the IBA-treated sterile anthers, the degeneration occurred at the pollen mother cell stage (Fig. 7) or the microspore stage (Fig. 8). At the later stages, the tapetum became slightly stretched, but its cells remained intact. Endothecial cells in such male steriles were generally devoid of fibrous thickenings.

Anthers developed normally in the coumarin-treated male steriles up to the pollen mother cell stage. At the tetrad stage, the tapetum cells were larger and healthier in treated plants than in the control. But the microspores developed vacuolation soon after their separation from the tetrads and showed signs of degeneration, while the tapetum layer remained intact (Fig. 9). The tapetum also degenerated at a later stage, and degenerated microspores and remains of tapetal cells were observed in mature anthers. Besides their persistent nature, the tapetal cells in some of the coumarin-induced male steriles also showed unusual enlargement (Fig. 10).

The vascular supply of the anthers was also altered in the chemically-induced male steriles. In the anthers from normal untreated plants, the vascular bundle in the connective was quite conspicuous and consisted of a healthy mass of vascular tissues (Fig. 11). The size of the vascular bundle in the IBA-and coumarin-induced male sterile anthers, however, was greatly reduced and the vascular tissue was poorly developed (Figs. 12 and 13). In MH-induced male sterile anthers, the vascular bundle was nearly normal in size, but the vascular tissues appeared to be degenerating (Fig. 14).

Discussion and Conclusions

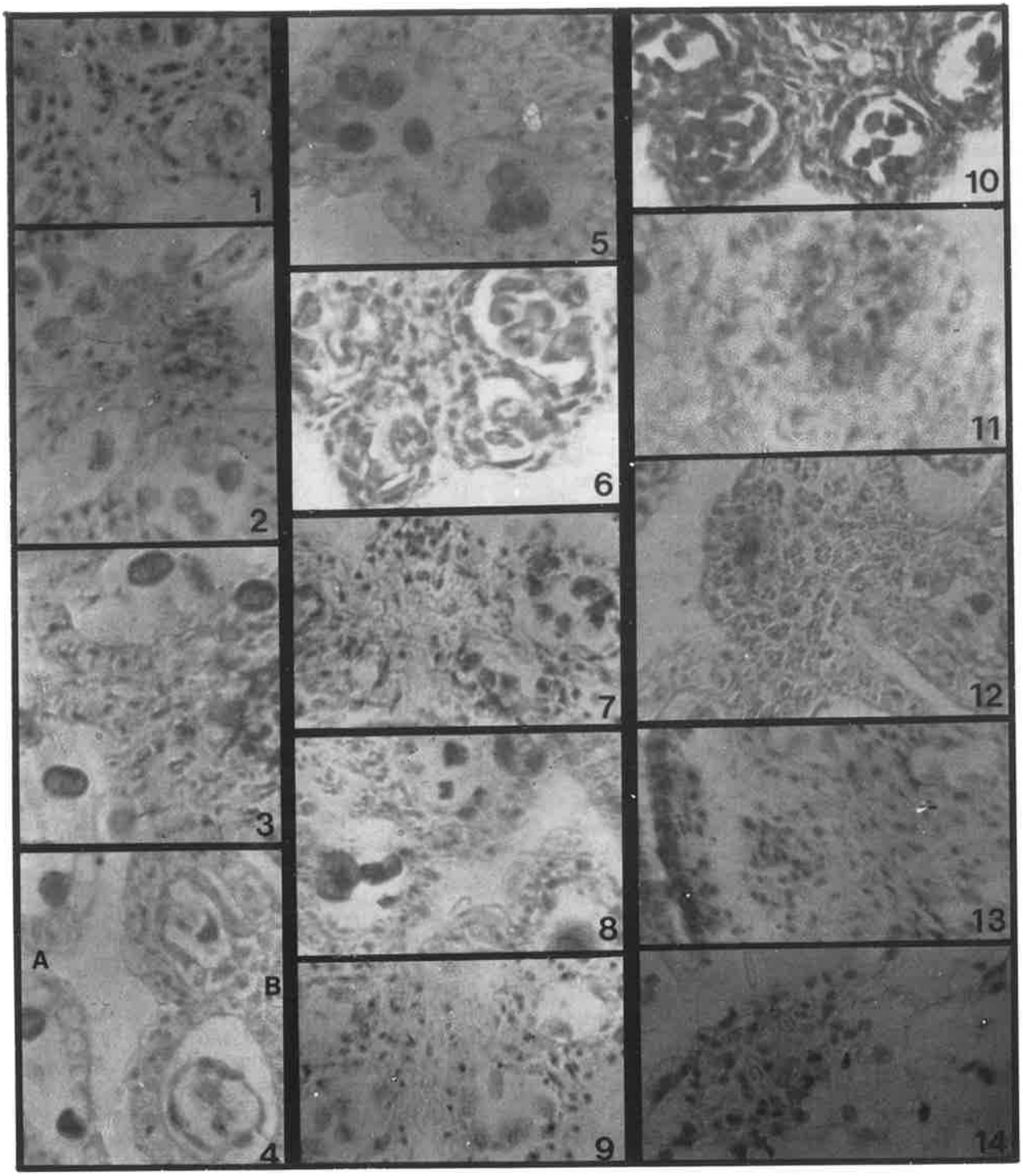
In chemically-induced sterile plants, a persistent or unusually enlarged tapetum was associated with delayed generation, which led to pollen abortion. A review of a number of plant species (Chauhan and Kinoshita 1982) also showed an association between chemically-induced male sterility and a malfunctioning tapetal layer.

The persisting tapetum apparently starves the developing microspores of nourishment. This process has been observed in MH-induced male sterile plants of *Sesamum indicum* (Kumar 1963); wheat (Hoagland *et al.* 1953); coriander, fennel, tobacco, cotton, and okra (Dubey 1968; Dubey and Singh 1969); and *Ranunculus muricatus* (Chauhan and Kumar 1980).

Besides its persistance, the tapetum showed unusual enlargement in some of the coumarin-induced male sterile anthers. This enlargement may indicate that nutrients are being witheld in these cells. Similar enlargements have also been noted in a number of other plant species rendered male sterile through chemical treatments, including: *Cajanus cajan*, *Phaseolus aureus*, *p. mungo*, *Trigonella foenum-graecum*, *Vicia faba* (Kaul 1968), *Cucumis melo*, *C. sativus*, *Luffa cylindrica*, *Momordica charantia*, and *Capsicum annuum* (Chauhan 1976; 1979 a and b; 1980).

The indehiscent nature and lack of endothecial thickenings in induced male sterile anthers noted in this study conform with the findings of Chauhan (1980b) in *Capicicum annuum*. Chauhan and Kumar (1980) in *Ranunculus muricatus*, and Dubey and Singh (1969) in coriander.

The findings of Painter (1943), Cooper (1952), Chang (1954), Takates (1962), and Chauhan (1963) have demonstrated that a proper supply of carbohydrates, DNA, or DNA precursors from the tapetum is imperative for microspores to develop properly into viable pollen grains. The findings in this paper as well as other studies show that gametocidal compounds primarily change tapetal cells; this change upsets the regular supply of nutrition to the sporogenous tissue and microspores, and the latter abort or develop into sterile pollen grains.



Figs. 1-14. Anther transverse sections of normal and chemically induced male sterile lentil. (1-5 and 11:control; 6 and 14:MH treated; 7,8,12,IBA treated; 9,10,13 coumarin treated); 1. Differentiated wall layers and sporogenous cells; 2. Mature anther showing endothecial thickenings and stretched epidermal layer; 3. A mature anther lobe showing lack of endothecial thickenings at the junction of two anther sacs; 4. Portions of two anthers of a flower showing markedly different stages of development, one with mature pollen grains (A) and the other with uninucleate pollen mother cells (B); 5. Mature anther showing confluence of anther sacs due to dissolution of partioning wall layers; 6. Degenerating pollen tetrads; 7. Degenerating pollen mother cells; 8. Degenerating microspores; 9. Microspores liberated from mother wall and showing signs of degeneration, tapetum layer intact; 10. Degenerating pollen grains and unusually enlarged tapetal cells; 11. Vascular bundle in the connective of control anther with conspicuous and healthy vascular tissues; 12-14. Abnormal vasculature in the anther connectives of chemically-treated plants.

(All figures x 400)

The chemical treatments also induced poorly-developed vascular tissue in the anther connectives, leading to a smaller vascular bundle or degenerated vascular tissues. Similarly-inhibited vascular development has been recorded in a number of plant species by Chauhan (1980) and Chauhan and Kinoshita (1982). They believe that reduced vascularity keeps nutrients from passing into various parts of the anther, including the tapetum- which becomes malformed, and the developing microspore- which aborts. Our study supported this view.

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Effects of some phytogametocides on growth, fertility, and yield of lentil

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Abstract

The effects of naphthalene acetic acid (NAA), indole butyric acid (IBA), maleic hydrazide (MH), and coumarin on vegetative growth, flowering, pollen sterility, fruit set, and seed yield were studied in lentil cultivars Pant-406 and JLS-2. Plant growth in general was retarded; the height and number of leaves in mature plants were significantly reduced in all treatments, while branching was enhanced. Yield and yield components-number of pods/plant and number of seeds/pod-were also significantly low in all the treatments. Floral abnormalities such as open flowers with protruding anthers and stigma, reduced size of flower, and flowers with (5) + (4) stamen configuration were also induced by some of the chemicals. Pollen grains aborted completely in Pant-406 treated with 0.1% IBA, and in JLS-2 treated with 0.07% MH; these treatments also induced 40-60% ovular abortion in the treated plants. Male

sterility induced by the treatments was also manifested in *in situ* pollen germination, *in situ* exudation of pollen cytoplasm, and modification of certain stamens into staminodes.

Introduction

Chemicals such as aliphatic acids or their derivatives, auxins, anti-auxins, and gibberellins have been tested for their phytogametocidal action on crop plants. The exhaustive literature on the effects induced by these chemicals on various crops has been reviewed by Jain (1959), Mohan Ram and Rustagi (1966), and Chauhan and Kinoshita (1982). However, since there are no reports on how these chemicals affect lentil (*Lens culinaris* Med.), this study investigated the effects of some chemicals on two lentil varieties.

Materials and Methods

Lentil cultivars Pant-406 and JLS-2 were grown in a randomized complete block design with four replications. Foliar sprays of aqueous solutions were applied for NAA at concentrations of 0.05, 0.075, and 0.1%; for IBA at 0.05, 0.1, and 0.25%; for MH at 0.075, 0.1, and 0.25%; and for coumarin at 0.5, 1.0, and 2.0%. Plants were sprayed at floral bud initiation (T_1) and one and two weeks later (T_2) and (T_3). Certain rows were sprayed only once; i.e., at the T_1 or T_2 stages, while others were sprayed twice, either at T_1 or T_2, T_1 or T_3, or T_2 and T_3, making five different combinations of the times of application for every chemical concentration.

Observations on (1) plant height, (2) number of branches/plant, (3) number of leaves/plant, (4) number of pods/plant, (5) number of seeds/pod, and (6) yield/plant were recorded at maturity and were analyzed statistically.

Pollen viability was tested in all the plants on the first day of flowering. In treatments which induced complete or appreciable pollen sterility, viability was tested every day thereafter, until considerable fertility was regained. Acetocarmine (0.5%) and iodine (0.5%) were used for the stain test of pollen viability.

For treatments in which total pollen sterility was indicated by the stain test, a field test of pollen viability was also made. This test provided an index of pollen viability using the percentage of seed set/fruit on untreated plants, after pollinating emasculated flowers with pollen from treated plants.

To determine the effect of the chemicals on ovular or female fertility, emasculated flowers of the treated plants showing complete pollen sterility were pollinated with pollen from untreated plants. The number of seeds/25 randomly selected pods was then compared with the seed set obtained from 25 control fruits.

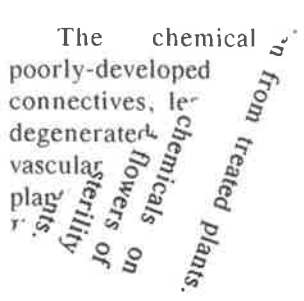
Results and Discussion

Growth and morphological characters

Phytotoxic effects–curled or twisted branches, burned or abscised leaflets–were induced by all four chemicals in both lentil cultivars. The severity of these effects varied in different chemicals, and increased with the concentration and number of applications. Similar effects from these chemicals have been recorded in a wide variety of crop plants (Mohan Ram and Rustagi 1966; Chauhan and Kinoshita 1982).

The effect of chemical treatments on various vegetative and reproductive parameters of growth is summarized in Table 1. A common feature of the treatments was that the height of treated plants was significantly less than the checks, because of the recession or death of the apical meristem. In both cultivars, MH reduced height the most, followed by IBA, NAA, and coumarin. The degree of reduction was also proportional to the concentration and number of applications. Growth inhibition by MH treatment is described by Currier *et al.* (1950), (Beach and Leopold (1953), and Fischnich and Patzold (1954). Growth recession due to coumarin and IBA treatments also has been observed in a number of crops (Kaul and Singh 1967 a and c; Dubey and Singh 1968; Bharadwaj and Dubey 1975, 1977). However, in contrast to our findings, Bharadwaj and Dubey (1975, 1977) reported that NAA did not inhibit the plant height in black gram and green gram.

Although the treatments decreased height, all treatments resulted in a positively significant increase in the number of branches and the treated plants were bushy in appearance. Checked apical growth leading to increased secondary branching is a common feature of MH and coumarin treatments (Mohan Ram and Rustagi 1966; Chauhan and Kinoshita 1982). Increased branching from treatment with NAA and IBA has also been observed in black gram and green gram (Bharadwaj and Dubey 1975, 1977).



The chemical ... the chemicals ... from treated plants ... poorly-developed ... eaves. Coumarin ... connectives, le... , while NAA reduced ... degenerated ... vascular ... so affected the root ... plan... ly pulled out from some ... and then studied. The ... nally consists of a strong ... ateral branches. There ... more ... in the NAA treatments, and ... and coumarin treatments. Experiments o... laudhary and Bhatnagar 1953), maize, oat (Carlson ...54), pea (Audus and Tresh 1956), cotton, garlic, and sugarbeet (Narayanswami 1960) have shown that MH retards root growth. Carlson (1954) reports complete absence of secondary roots in maize and oat plants receiving MH treatments, while Bharadwaj and Dubey (1975) observed that NAA increased the number of lateral roots in black gram. In their experiment, IBA checked the growth of primary roots and root growth continued only through secondary laterals.

Reproductive Growth

Flowering was delayed by NAA, IBA, and MH treatments, but unaffected by coumarin. Delayed flowering from MH treatment has been reported in bristal raspberries (White 1950) and corn (Josephson 1951) and from coumarin in apricot (Soni and Yousif 1978). Floral abnormalities such as open flowers, with anther and stigma protruding, were induced by NAA and IBA in JLS-2. Flowers in MH treated plants were smaller in size. In coumarin treatments, certain flowers showed a (5)+(4) stamen configuration.

Table 1 also summarizes the effect of the chemical treatments on reproductive growth of the plants, measured in terms of number of pods/plant, seeds/pod, and total seed yield/plant. The data reveal that all three productivity parameters were significantly influenced by the treatments.

MH proved to be the most injurious to pod setting in both varieties, while IBA produced the highest pod set, followed by NAA. MH also inhibited seed set the most, whereas the highest seed set was recorded in coumarin treatments in Pant-406. In terms of seed yield, IBA produced the highest yield in JLS-2 and NAA did the same in Pant-406.

The yield components decreased in both varieties as chemical concentrations increased. Markedly reduced fruit and seed set, as well as total seed yield/plant, have been common in a wide variety of plant species subjected to gametocidal treatments (Chauhan and Kinoshita 1982; Moore 1950; Josephson 1951; Foy and Miller 1963; Kumar 1963; Kaul 1968; Dubey and Singh 1968; and Kaul and Singh 1967 a, b, and c). In contrast to our findings, Bharadwaj and Dubey (1975, 1977) reported that NAA and IBA treatments did not affect the seed set/fruit in black gram and green gram, but the pod set and total yield were reduced in their experiment.

The yield of seeds/plant is reduced because of ovular sterility, abscission of flowers, inadequate pollination or fertilization, or other histological causes. The abscission of flowers or flower buds is caused by the phytotoxicity of the gametocidal compounds, as reported in *Cajanus* (Kaul and Singh 1967a), sesame (Kumar 1963), cotton (Singh 1964), and tobacco (Jos 1964). Inadequate pollination is also responsible to some extent for reduced yield, because a greater or moderately greater amount of fruit is set in various treatments at the time of complete male sterility if the flowers are hand pollinated. The higher yield from hand pollinated flowers shows that inadequate pollination, due to the arrest of the stigma inside the keel petal in leguminous plants, also contributes to lower yield. At times, the morphological variations produced in the flowers by gametocidal compounds, such as staminode formation or weakened filaments of the anther, also limit pollination.

Pollen Sterility

The pollen grains in the normal untreated plants of the two varieties were fully turgid and were stained uniformly by acetocarmine or iodine. Varying degrees of pollen sterility were induced by all four chemicals in the two lentil varieties (Table 2). A few treatment combinations induced complete pollen abortion; all such pollen grains were devoid of any cytoplasmic content and failed to pick up the stain. Other manifestations of pollen sterility were *in situ* pollen germination, *in situ* exudation of pollen cytoplasm, and modification of certain stamens (one-seven) into staminodes.

Various chemicals differed significantly in their ability to induce pollen sterility in the two varieties. The greatest pollen sterility was induced by NAA in JLS-2 and by IBA in Pant-406, while MH treatments induced less pollen sterility in both varieties.

The degree of induced pollen sterility has been shown to be directly proportional to the

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The severe phytotoxic effects of the chemicals decreased the number of leaves in the treated plants through abscission of some of the leaves. Coumarin treatments reduced the leaf number, while NAA reduced it further.

The chemical treatments also affected the root system. The roots were gently pulled out from some plots after heavy irrigation, and then studied. The lentil's root system normally consists of a strong primary root with slender lateral branches. There were fewer root laterals in the NAA treatments, and more in the IBA, MH, and coumarin treatments. Experiments on onion (Chaudhary and Bhatnagar 1953), maize, oat (Carlson 1954), pea (Audus and Tresh 1956), cotton, garlic, and sugarbeet (Narayanswami 1960) have shown that MH retards root growth. Carlson (1954) reports complete absence of secondary roots in maize and oat plants receiving MH treatments, while Bharadwaj and Dubey (1975) observed that NAA increased the number of lateral roots in black gram. In their experiment, IBA checked the growth of primary roots and root growth continued only through secondary laterals.

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The degree of induced pollen sterility has been shown to be directly proportional to the

Table 1. Effect of different concentrations of chemicals on certain characteristics in lentil cultivars JLS-2 and Pant-406*.

Chemical	Concentration (%)	Plant height (cm)	Number of branches	Number of leaves	Number of pods/ plant	Number of seeds/ pod	Seed yield/ plant (g)
NAA	0.05	26.2	16.3	106.5	64.0	1.81	1.2
		(33.10)	(42.0)	(530.5)	(364.5)	(1.56)	(4.1)
	0.075	26.0	10.8	78.0	98.0	1.34	1.3
		(32.6)	(56.7)	(406.0)	(349.0)	(1.46)	(2.7)
	0.1	15.3	7.6	72.5	40.5	1.03	1.0
		(30.6)	(61.0)	(358.0)	(297.0)	(1.06)	(2.6)
IBA	0.05	30.5	22.8	228.0	166.5	1.35	1.8
		(35.0)	(55.9)	(438.0)	(381.0)	(1.47)	(4.6)
	0.1	29.2	1.0	205.0	159.0	1.11	1.5
		(33.7)	(60.0)	(431.5)	(367.5)	(1.33)	(2.3)
	0.25	27.7	16.6	162.5	154.5	1.10	1.3
		(32.8)	(61.8)	(409.0)	(331.5)	(1.08)	(1.4)
MH	0.075	24.1	15.2	119.0	75.8	1.70	1.0
		(32.4)	(36.1)	(256.5)	(274.5)	(1.30)	(3.1)
	0.1	-	-	-	-	-	-
		(29.6)	(36.3)	(223.0)	(260.0)	(0.93)	(2.1)
	0.25	-	-	-	-	-	-
		(13.1)	(16.5)	(136.5)	(-)	-	(-)
Coumarin	0.5	31.8	19.3	107.0	75.8	1.89	1.2
		(34.6)	(35.7)	(324.5)	(235.5)	(1.49)	(3.7)
	1.0	29.3	17.3	96.3	52.4	1.43	1.1
		(34.4)	(40.5)	(286.0)	(234.8)	(1.40)	(2.9)
	2.0	21.3	14.5	61.5	51.1	1.52	1.0
		(33.6)	(28.3)	(268.0)	(210.0)	(0.99)	(1.6)
Control	0.0	40.0	12.5	260.0	142.5	2.00	8.3
		(40.0)	(18.3)	(750.0)	(575.0)	(2.00)	(9.5)
L.S.D. (5%)		3.5	5.0	45.6	37.7	0.02	0.3
		(2.7)	(9.9)	(87.7)	(71.0)	(0.47)	(0.7)

*Pant-406 values are in parentheses

concentration of the chemicals utilized, a relationship found in a wide variety of crop plants on which gametocidal chemicals have been tried (Mohan Ram and Rustagi 1966; Chauhan and Kinoshita 1982).

Out of the two single spray treatments, the treatment given one week after the bud initiation (T_2) was the most effective in causing maximum pollen sterility. Out of the three double application treatments, the treatment given at the time of bud initiation followed by another application after a week (T_1T_2) or two weeks (T_1T_3) was the most effective at inducing maximum pollen sterility. Similar effects from the number and timing of sprays have been found by Kumar (1963), Singh (1964), Kaul and Singh (1967a), and Dubey (1968) in other crops.

A concentration of 0.075% MH induced complete pollen sterility in JLS-2; 0.1% IBA did the same in Pant-406. MH also induced such sterility in sorghum

Table 2. Effect of different concentrations of NAA, IBA, MH, coumarin, and time of application on pollen sterility percentage in lentil cultivars JLS-2 and Pant-406*

Chemical	Concentration (%)	Time of application				
		T_1	T_2	T_1T_2	T_1T_3	T_2T_3
NAA	0.05	60.0 (28.0)	45.0 (21.0)	30.0 (15.0)	60.0 (30.0)	50.0 (25.0)
	0.075	70.0 (-)	45.0 (55.0)	50.0 (38.0)	25.0 (35.0)	40.0 (-)
	0.1	30.0 (30.0)	40.0 (-)	30.0 (-)	45.0 (50.0)	20.0 (-)
IBA	0.05	40.0 (26.0)	15.0 (30.0)	25.0 (46.0)	60.0 (78.0)	60.0 (60.0)
	0.1	15.0 (48.0)	70.0 (35.0)	40.0 (100.0)	50.0 (70.0)	70.0 (80.0)
	0.25	26.0 (25.0)	20.0 (69.0)	10.0 (76.0)	30.0 (73.0)	90.0 (41.0)
MH	0.075	30.0 (56.0)	30.0 (20.0)	100.0 (33.0)	35.0 (85.0)	20.0 (39.0)
	0.1	- (-)	30.0 (-)	50.0 (-)	30.0 (-)	10.0 (32.0)
	0.025	- (-)	5.0 (-)	20.0 (-)	- (-)	16.0 (-)
Coumarin	0.5	40.0 (55.0)	25.0 (30.0)	15.0 (20.0)	15.0 (25.0)	99.9 (35.0)
	1.0	20.0 (10.0)	10.0 (25.0)	45.0 (30.0)	100.0 (35.0)	- (30.0)
	2.0	70.0 (28.0)	80.0 (25.0)	30.0 (40.0)	50.0 (35.0)	45.0 (30.0)
Control		2.0 (2.0)				
L.S.D. (5%)		8.9 (13.3)				

*Values for Pant-406 are in parentheses.

(McIlrath 1953), okra, coriander, kenaf, fennel (Dubey 1968), datura, and chillies (Chauhan and Singh 1972); while IBA did so in cluster bean and eggplant (Awasthi and Dubey 1982, a and b). NAA and coumarin failed to cause complete pollen abortion in either lentil variety. Similarly, NAA sprays failed to cause complete feminization in black gram and green gram (Bharadwaj and Dubey 1975, 1977) and in corn (Krishnamoorthy and Talukedar (1979).

The plants in treatments which induced total pollen sterility remained sterile for a certain period after which they gradually regained fertility. The total pollen sterility lasted 15 and 16 days in the two lentil varieties. In treatments for which the stain test revealed the induction of total pollen sterility, the fertilizing ability test for the pollen grains showed that no control flowers pollinated with the treated (100% sterile) ones set

any viable seeds. Some seedless pods, however, were induced after artificial pollination with the sterile pollen.

Ovular Sterility

In the completely male sterile plants induced by MH and IBA, female sterility was tested by pollinating the flower buds with pollen from the control. MH induced 40-60% ovular sterility along with complete pollen sterility. Similarly, in cotton (Singh 1964) and sesame (Kumar 1963; Chauhan and Singh 1971), MH has been found to reduce the amount of seed set in treated plants.

IBA treatments also reduce female fertility. The percentage of seed set in treated male sterile plants is reduced to nearly 40-60% of the control. However, in contrast to our findings, Bharadwaj and Dubey (1975, 1977) found that the number of seeds/pod of black gram and green gram were unaffected by IBA treatments.

In conclusion, it may be pointed out that none of the chemicals tested gave promising results, as the seed set was drastically reduced in all treatments which induced male sterility. Further experimentation is required to elucidate the correct concentrations and timing of applications of MH and IBA. A serious handicap to commercial production of hybrid lentil seed is the arrest of the stigma inside the keel petals. When all the inner whorls of a flower are unexposed, these male sterile flowers are unable to set seed when naturally cross-pollinated. Hand exposure is too costly and time-consuming to be used commercially. However, NAA and IBA treatments which produce some modified flowers show promise for further experimentation.

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A chance trisomic in the progeny of an interchange heterozygote in lentil

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Abstract

Two trisomic plants were found in the progeny of an interchange heterozygote. They had chromosome counts at meiosis of 2n = 15. Two chromosomes were very small in size and were usually present as univalents and rarely as bivalents. In some cells a trivalent was seen, confirming the trisomic nature of the plants.

The production of a trisomic series in a diploid crop like lentil is a prerequisite for any systematic work on location of genes on specific chromosomes. In a research program involving cytogenetic studies in lentil, we have been trying to produce both a complete translocation tester set and a trisomic series. While progress has been made on producing a translocation tester set, our efforts to produce a trisomic set were futile. To produce trisomics, we produced a number of tetraploids using aqueous colchicine solutions (Gupta and Singh 1982). Although these tetraploids are being maintained regularly, no success was achieved in 4x x 2x crosses to produce triploids for trisomic production. However, a chance trisomic was available in the progeny of an interchange heterozygote (Gupta and Singh 1982; Sharma *et al.* 1983). Since this triploid was sterile, it could neither be used for trisomic production nor be maintained. While we plan to use the embryo rescue technique to make successful 4x x 2x crosses, this year we came across a chance trisomic in the progeny of an interchange heterozygote, which is described here. Two trisomic plants were available in the progeny of an interchange heterozygote designated T35 (1)-7(1). The plants were normal in appearance, although the seeds were lighter in color and smaller in size than usual.

Table 1. Metaphase I configurations in a trisomic lentil plant (2n = 15).

Chromosome associations (Means; range)				
I	II		III	IV
	ring	rod		
0.53 (0-2)	3.4 (0-5)	2.4 (0-4)	0.27 (0-1)	0.20 (0-1)

Table 2. Chromosome distributions at anaphase I in a trisomic lentil plant (2n = 15).

Number of cells	Distribution
8	7:8
3	7:1:7
1	5:2:8

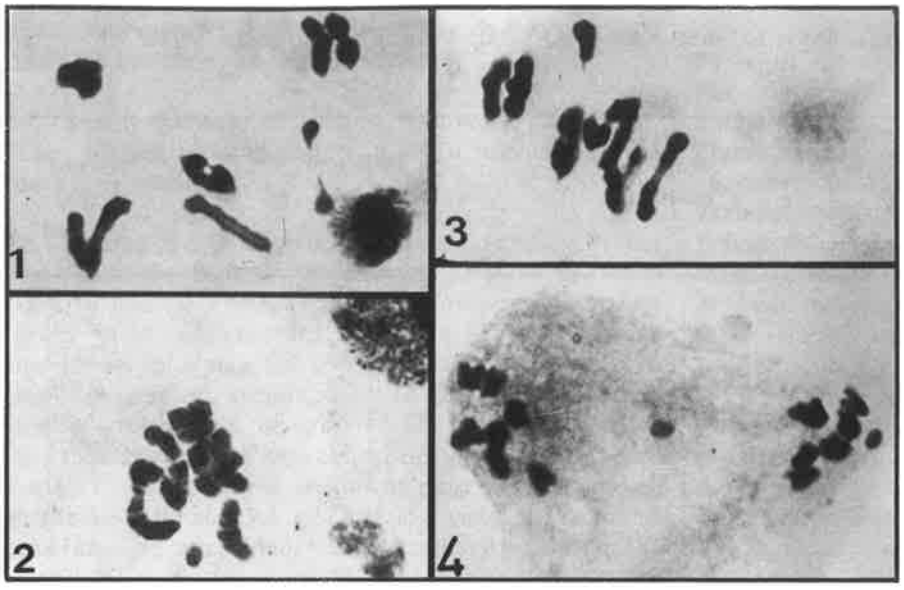

Fig. 1. Metaphase I showing 6″ + 1″. Figs. 2 and 3. Metaphase I showing a pentavalent in each case. Fig. 4. Anaphase I showing 7:1:7 distribution.

At meiosis, these plants had a chromosome count of 2n = 15; two of these chromosomes were very small in size and usually present only as univalents, although they were in rare cases associated in a bivalent. There was also a distinct trivalent in a number of cells, confirming the trisomic nature of these plants (Fig. 1). A quadrivalent or a pentavalent was also infrequently present, suggesting that interchange heterozygosity existed (Figs. 2 and 3; Table 1). The presence of a pentavalent also suggested that the plants were trisomic for one of the chromosomes involved in the interchange. In addition, at anaphase I, 2n = 15 chromosomes could be counted easily (Table 2), and one of these chromosomes was sometimes present as a laggard (Fig. 4).

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Performance of bold-seeded lentil varieties in calcareous soil of North Bihar region

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Abstract

The performance of 14 bold-seeded and one small-seeded lentil variety was examined in sandy loam and calcareous soil at Dholi, North Bihar, India in the 1984/85 season. The variety Sehore-34 was the earliest to mature (105 days) followed by Sehore-74-3 (107 days), and Sehore-74-7, BR-25, and JL-1 (110 days each). Among the bold-seeded varieties tested, Precoz Sel had the heaviest seeds (4.59 g/100 seeds) followed by JL-1 (4.12 g/100 seeds). The lowest 100-seed weight (2.69 g) was recorded in variety L 4076, followed by BR-25 (2.92 g) and Pant L-77-2 (2.88 g). In general, varieties with lower seed weight gave higher grain yields. Variety L-4076 gave the highest yield of 2770 kg/ha, followed by Pant L-77-2 (2375 kg/ha) and BR-25 (2354 kg/ha). The small-seeded check variety Pant L-639 yielded similarly to Pant L-77-2. In the bold-seeded lentil, negative association was also recorded between seed weight and grain yield.

Introduction

Lentil is an important *rabi* pulse crop in Bihar, occupying 164,000 hectares with an annual production of 112,000 metric tonnes. The lentil *microsperma* subspecies grown in India are subdivided into two groups according to seed size: small-seeded and bold-seeded. Bold-seeded lentils generally yield less than small-seeded ones. All varieties released nationally thus far have been small-seeded types. Recently, the lentil breeders, participating in the

Table 1. Days to 50% flowering, days to maturity, 100-seed weight, and grain yield of lentil varieties.

Variety	Days to 50% flowering	Days to maturity	100-seed weight (g)	Grain yield (kg/ha)
L-4076	69	133	2.69	2770
Pant L-77-2	68	131	2.88	2375
Pant L-639	71	127	1.53	2375
BR-25	63	110	2.92	2354
WBL-58	57	118	3.42	1791
L-4162	69	138	3.37	1500
L-4163	73	138	3.45	1500
LG-170	72	137	3.02	1311
Sehore-74-7	58	110	3.52	1208
JL-1	65	110	4.12	1186
Sehore-34	58	105	3.45	1166
WBL-12	54	115	3.51	1061
LG-171	74	139	3.13	916
Sehore-74-3	58	107	3.49	895
Precoz Sel	63	125	4.59	686
G.M.	64.8	122.9	3.28	1539
L.S.D. at 5%				255.6
C.V.				12.06%

All Indian Coordinated Pulses Improvement Workshop decided to test the bold-seeded varieties separately. Bold-seeded lentils are preferred by consumers and have a higher market price. Bold-seeded lentils are also considered more tasty when prepared as "dal." Accordingly, bold-seeded varieties with a 100-seed weight of more than 2.5 g, collected from different research centers in India, were tested at the College Research Farm, North Dholi, India.

Materials and Methods

Fourteen bold-seeded lentil varieties were evaluated against a small-seeded high yielding variety, Pant L-639. The soil of the experimental plot was calcareous and sandy loam in texture, with a pH of 8.2, E.C. 0.53 mmhos/cm, organic carbon 0.56%, and 23 kg P_2O_5/ha; 215 kg/ha potash; and 32% free $CaCO_3$. The initial Zn status of the soil was 0.38 ppm, and initial Fe status was 6.47 ppm. Soil analysis showed that the soil was deficient in organic carbon, P_2O_5, Zn, and Fe.

The experiment was conducted in a randomized complete block design with four replications. Sowing was done in rows spaced 30 cm apart on 14 Nov 1984. The plot size was 6.0 m^2, with 5 rows, each 4 m long. Observations were recorded on days to 50% flowering, days to maturity, 100-seed weight, and grain yield/plot.

Results and Discussion

Data on yield and other characteristics are given in Table 1. As shown, days to 50% flowering ranged from 54 to 73 days. Although variety WBL-12 from West

Bengal was the earliest to flower, it was not the earliest to mature. LG 171 from Punjab flowered and matured the latest, among the varieties.

Sehore-34 was the earliest to mature (105 days). The highest yielding variety, L-4076, matured in 133 days after sowing.

Among the bold-seeded varieties tested, Precoz Sel, had the highest 100-seed weight (4.59 g), while L-4076 had the lowest (2.69 g). However, the highest grain yield was recorded in L-4076 (2770 kg/ha) and the lowest in Precoz Sel (686 kg/ha). The second highest yielding variety, Pant L-77-2 was the second lowest in 100-seed weight (2.88 g), indicating a negative correlation between grain yield and 100-seed weight. The 100-seed weight of most varieties ranged between 3.0 and 3.5 g. The low yield of Precoz Sel can also be attributed to its sensitivity to zinc and iron deficiency. Variety, JL-1 had the second highest 100-seed weight (4.12 g) and a medium grain yield.

Incidence of black seed coat in lentil

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Abstract

The seed coats of three lentil cultivars were examined for pigmentation. The two small-seeded cultivars had 47.5 and 56.0% black seeds, while the large-seeded cultivar has only 5.0%.

Introduction

In lentil, there is a problem with the variation of the color of the seed coat; it can vary within a single plant. This study looked at the dark pigmentation of the seed coat in three lentil varieties: Pant L 639 and LL-78, small-seeded varieties with 100-seed weights less than 2 g; and Sehore 74-7, with about 3 g/100-seed weight. All three varieties belong to the *microsperma* sub-species of *Lens culinaris*. Other grain characteristics of these varieties are given in Table 1.

These varieties were grown during the *rabi* (November-April) season of 1983/84. Five hundred plants were randomly selected from each variety to study seed coat pigmentation; observations are presented in Table 2.

Table 1. Grain characteristics of the three lentil varieties.

Variety	Average number of seeds/ plant	100-seed weight (g)	Seed coat color	Coty-ledon color
Pant L 639	200	1.60	Grey mottled	Red
LL-78	200	1.75	Grey mottled	Red
Sehore 74-7	80	3.00	Grey mottled (deep mottling)	Red

Table 2. Percentage of seeds with black seed coat.

Variety	Number of plants observed	Number of plants with black seeds	Percentage of plants with black seeds	Percentage of black seeds in a plant (range)
Pant L 639	500	237	47.5	1-30
LL-78	500	280	56.0	0.5-50
Sehore 78-7	500	25*	5.0	2-8

*Seed coat not completely dark.

In the small seeded varieties, Pant L 639 and LL-78, the percentage of plants with a black seed coat was greater than in the bold-seeded variety. The percentage of black seeds in a plant was also higher in the small-seeded varieties. It was further observed that the pigmentation of the seed coat in the bold-seeded variety was not as dark as in the small-seeded varieties, although Sehore 74-7 has inherently darker mottling compared to Pant L 639 and LL-78.

Vaillancourt and Slinkard (1983) reported that black seed coats are generally high in tannin content. They found 0.0-1.4% tannin content in the seed coats of different lentil varieties. Tannins endow plants with resistance to stress; however, a lower level is necessary for legumes to be accepted by consumers, as suggested by Hewitt and Ford (1982).

Black seed may contain slightly more tannin; the deposition of this substance in the seed coat varies from plant to plant and even from seed to seed in a plant. But breeders should develop a variety without black seeds, since these bring a lower market price. Climatic and nutritional factors should also be studied in relation to the development of the black seed coat.

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Studies on variability in lentil germplasm in Madhya Pradesh, India

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Abstract

Five-hundred lentil accessions were evaluated for time to flowering, maturity, plant height, 100-seed weight, seed yield, and disease incidence in Madhya Pradesh, India. Ranges in variation for various characteristics were: time to flowering, 55-108 days; time to maturity, 118-160 days; plant height, 17.8-39.6 cm; 100-seed weight, 1.0-4.9 g; and seed yield, 5-180 g/m^2.

Introduction

Lentil (*Lens culinaris* Medik.) is second in importance only to chickpea as a rabi pulse crop in India; the country also accounts for almost 50% of world lentil production. In India, Madhya Pradesh is an important lentil growing state. In Madhya Pradesh, the area under lentil increased from 273,000 hectares in 1980 to 309,000 hectares in 1983, and production increased from 108,000 metric tonnes in 1980 to 121,000 metric tonnes in 1983. The state's important lentil-growing districts are Sagar, Raison, Vidieha, Dameh, Bhind, and Seoni. Only bold-seeded lentils are grown by farmers. In general, lentil yield is low (average 390 kg/ha), but is higher in Raison (557 kg/ha) and Dameh (425 kg/ha), and the highest in Sehore (597 kg/ha) and Bhopal (589 kg/ha) (Anon. 1983).

Maintaining and evaluating economic traits of germplasm is an important prerequisite in a crop improvement program. This paper summarizes an evaluation of lentil germplasm in Madhya Pradesh.

Materials and Methods

Five-hundred lentil accessions were grown at the Experimental Research Farm of the Main Pulse Research Centre at Rafi Ahmed Kidwai College of Agriculture, Sehore, Jawaharlal Nehru Agricultural University Campus, Madhya Pradesh. The experiment was sown on 20 Oct 1984 in an augmented design using two checks, Sehore-34 and Sehore-74-3, after every 25 accessions. The accessions included 103 from the National Board for Plant Genetic Resources (NBPGR), 68 from Uttar Pradesh, 47 from West Bengal, 2 each from Bihar, Maharashtra, and Punjab, five from Delhi, and 271 from different regions of Madhya Pradesh. Each plot was a single 4m-long row, spaced 25 cm apart from neighboring rows. Fertilizer was applied at 100 kg diammonium phosphate (18% N and 46% P_2O_5)/ hectare as a base application. The crop was not irrigated and no rain fell during the growing period.

Germplasm was evaluated for flowering (days), maturity (days), plant height (cm), 100-seed weight (g), seed yield (g/m^2), and disease incidence.

Table 1. Meteorological data.

Month	Temperature (°C) Max.	Min.	Rainfall (mm)	Relative Humidity (%)
Nov 1984	28.86	9.87	0	51.2
Dec 1984	27.90	8.20	0	61.1
Jan 1985	29.80	10.00	0	84.5
Feb 1985	27.20	6.80	0	58.5
Mar 1985	36.30	16.90	0	55.9
Apr 1985	39.70	19.61	0	47.8

Sehore is 499 m above sea level, latitude 27°12' N and longitude 77°05' E. The soils at the station are clay loam or medium black, low in available nitrogen, medium in phosphorus, and high in potash, with a pH of 7.8. The annual rainfall, 1000-1250 mm, comes mostly in August and September. Sehore has a sub-tropical climate. The meteorological data for the growing season of the crop are given in Table 1.

Results and Discussion

The range, mean, and coefficient of variation for the different characters studied are given in Table 2.

Time to flowering: This character was recorded on a line basis as number of days from seeding to flowering, for about 50% of the plants. The range was 55 days (SL-952) to 108 (SL-600).

Time to maturity: This character, also recorded on a line basis, was the number of days from seeding to the time when about 90-95% of the pods turned brown. The range was from 118 days (SL-10038, SL-992, and SL-281) to 160 days (SL-1054).

Plant height (cm): This character, recorded as the mean of five plants randomly selected from each row, ranged from 17.8 cm (SL-993) to 39.6 cm (SL-604).

Seed weight: The weight (g) of 100 seeds was recorded for seeds randomly selected from each row. The range was 1.0 g (SL-668) to 4.9 g (SL-712).

Seed yield: Seed yield (g/m^2) ranged from 0.5 g (SL-534 and SL-693) to 188 g (SL-598).

The seed size and seed yield of the accessions showed high variability which can be exploited in lentil improvement programs. Twenty-four lines yielded more than the check lines; that is, higher than 100 g/m^2. The seed size of these lines ranged from 2.3-4.2 g/100 seeds; four lines-SL-598, -904, -3, and -397-had the highest yields in the 1983/84 season (Anon. 1984).

Disease incidence: No disease appeared during the growing season, probably because of the drought conditions.

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Table 2. Range, mean, variance, and coefficient for five lentil characters.

Character	Range	Mean	Variance	Coefficient of variation (%)
Time to flowering (days)	55-108	74.0	94.0	13.1
Time to maturity (days)	118-160	134.0	86.6	6.9
Plant height (cm)	17.8-39.6	28.2	11.6	12.1
100-seed weight	1.0-4.9	3.0	0.45	22.4
Seed yield (g/m^2)	0.5-180	53.4	786.3	52.5

Genetic diversity in lentil

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Abstract

D^2 analysis was carried out on 56 lentil genotypes grown in a replicated trial. Analysis of variance revealed significant differences for all characters except plant height and branches. Fifty-six genotypes were classified into 13 clusters. Cluster IV had most genotypes (13) followed by clusters II and IX, which had 10 genotypes each. Maximum intercluster distance was observed between XI and XIII (13.18); with the next greatest distance between X and XIII (12.07). Of the characters studied, days from flowering to maturity contributed most to total genetic divergence (68.78%), followed by seed yield (20.57) and harvest index (17.66). Geographical diversity had no relation with genetic diversity. Thirteen genotypes were identified for further use in a lentil hybridization program.

Introduction

The inclusion of diverse genotypes in a hybridization program allows superior recombinations. There have been, however, only a few studies of genetic diversity in lentil germplasm collections (Tikka *et al.* 1977; Chandra *et al.* 1978; Singh 1982; Sapra *et al.* 1984). Among several methods of multivariate analysis for classifying germplasm collections, the D^2 analysis was found to be the best for assessing genetic diversity. The object of this study was to select diverse genotypes for a hybridization program aimed at improving seed yield.

Materials and Methods

Fifty-six lentil genotypes from different eco-geographical regions were sown on 21 Nov 1983 in a randomized complete block design with three replications. Each plot consisted of a single 3 m long row with 30 cm between plots and 5 cm between plants in a row. A border row was planted at the end of each replication. Ten competitive plants were selected from each plot to record time to flowering, time from flowering to maturity, time to maturity, plant height, branches, pods/plant, 100-seed weight, biological yield, harvest index, and seed yield.

Mahalanobis's D^2 analysis as outlined by Rao (1952) was used to assess the association among genotypes. Tocher's technique (Rao 1952) was used to group genotypes into different constellations. Canonical analysis (Arunachalam and Ram 1967) was carried out on the same data.

Table 1. Genotypes included in different clusters.

Cluster	Genotype
I	JPL20
II	JPL972, JPL534, JPL613, JPL952, Pant L639, JPL1058, JPL568, JPL403, JPL554, JPL927
III	JPL765, Pant L406
IV	JPL543, JPL953, JPL721, JPL982, JPL781, JPL120, JPL220, JPL610, JPL754, JPL50, JPL323, JPL389, JPL120
V	JPL495, JPL384
VI	JPL401, *Lens* 830, JPL923
VII	JPL256, JPL207, JPL306
VIII	JPL488, JPL611, JPL548, RAU101, JPL594, L9-830, L56
IX	JPL930, JPL530, JPL542, JPL1056, L9-12, JPL547, JPL511, JPL426, JPL510, K-L-1
X	JPL1059
XI	JPL1054, JPL1055
XII	JPL400
XIII	JPL983

Results and Discussion

The genotypes were significantly different from each other in all characters except plant height and number of branches. Genotypes were grouped into clusters using Tocher's technique; the composition of different clusters is given in Table 1. Cluster IV had the most genotypes (13) followed by clusters II and IX which had 10 genotypes each. Clusters I, XII, and XIII each had only one genotype.

The intra-and inter-cluster distances are presented in Table 2 and Fig. 1. The greatest intra-cluster distance was observed in IV (3.94) followed by XI (3.83), VIII (3.68), and XIII (3.67).

Cluster means for various characters are given in Table 3. The means for different clusters were appreciably different for the characters of seed yield, harvest index, biological yield, time from flowering to maturity, and seed size. Genotypes in Cluster I were tall, had more branches, and a high biological yield, harvest index, and seed yield. Cluster II had genotypes with late maturity and smaller seeds, while cluster V had bold-seeded genotypes. Genotypes in cluster VI were early maturing, and those in cluster VII had low biological yield. Clusters X and XI had late-flowering genotypes with the shortest time from flowering to maturity, while clusters XII and XIII had early-flowering genotypes and a long period from flowering to maturity. Genotypes JPL20, JPL1058, JPL765, JPL721, JPL495, *Lens* 830, JPL256, JPL548, JPL426, JPL1059, JPL1054, JPL400, and JPL983 were selected as the best parents for a hybridization program; these parents all belong to the different clusters.

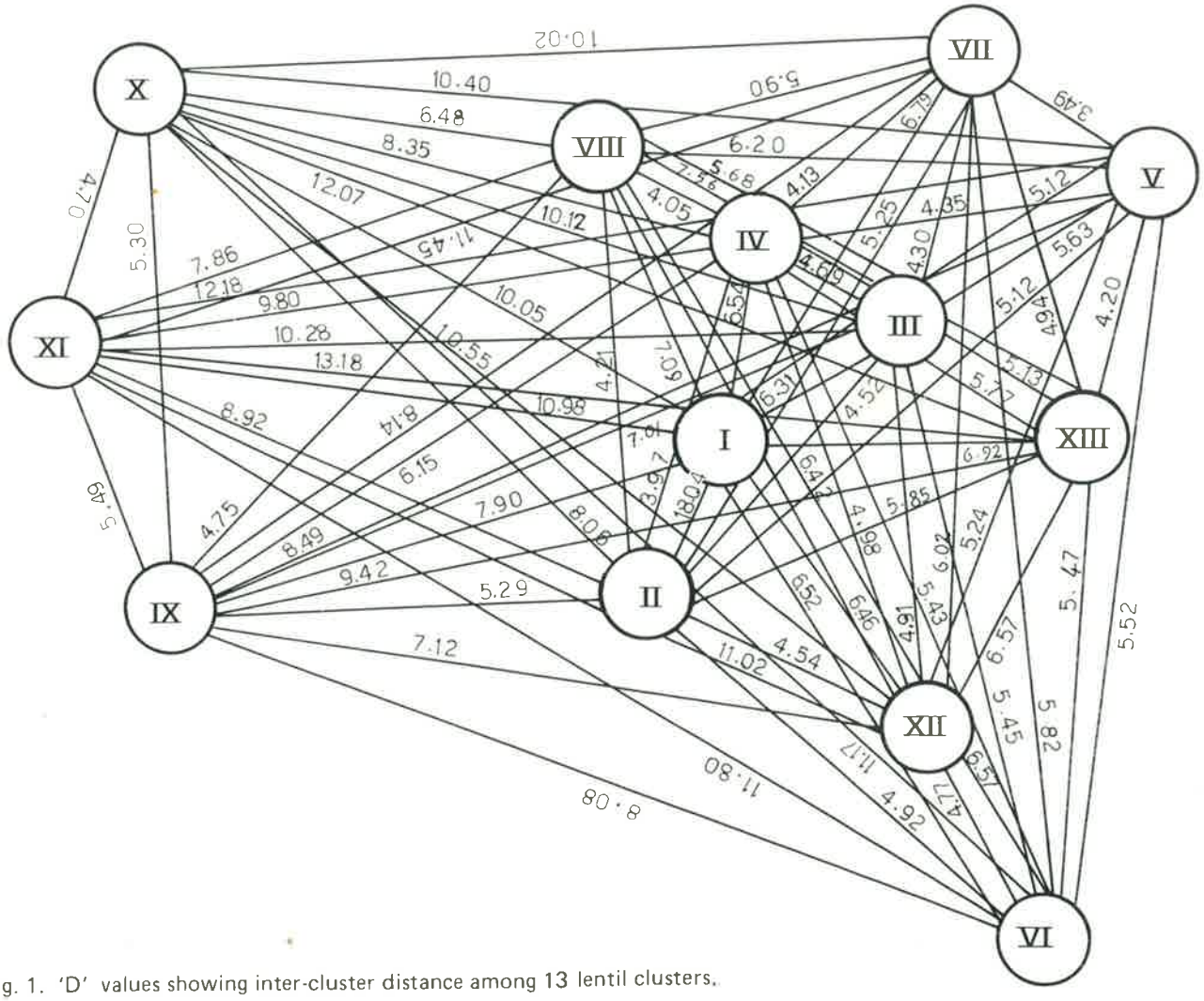

Fig. 1. 'D' values showing inter-cluster distance among 13 lentil clusters.

Table 2. Intra-and inter-cluster distance D^2 and D values.

Cluster	Total genotypes	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	1	00.00 (00.00)	32.55 (18.04)	39.85 (6.31)	42.84 (6.54)	31.80 (5.63)	42.63 (6.52)	46.20 (6.79)	50.33 (7.09)	62.49 (7.90)	101.17 (10.05)	120.67 (10.98)	29.83 (5.46)	47.86 (6.92)
II	10		10.90 (3.30)	20.43 (4.52)	15.81 (3.97)	26.29 (5.12)	24.22 (4.92)	27.56 (5.25)	17.76 (4.21)	27.47 (5.24)	65.07 (8.06)	79.60 (8.92)	20.66 (4.54)	34.32 (5.85)
III	2			9.60 (3.09)	22.03 (4.69)	26.21 (5.12)	29.76 (5.45)	18.53 (4.30)	32.25 (5.68)	49.20 (7.01)	102.41 (10.12)	105.78 (10.28)	24.17 (4.91)	26.40 (5.13)
IV	13				15.57 (3.94)	18.93 (4.35)	29.52 (5.43)	17.04 (4.13)	20.30 (4.05)	37.82 (6.15)	69.76 (8.35)	96.22 (9.8)	24.84 (4.98)	33.29 (5.77)
V	2					9.94 (3.15)	30.50 (5.52)	12.23 (3.49)	38.48 (6.20)	70.95 (8.49)	108.17 (10.40)	148.49 (12.18)	27.46 (5.24)	17.66 (4.20)
VI	3						12.14 (3.48)	33.95 (5.82)	43.27 (6.57)	65.44 (8.08)	124.78 (11.17)	139.40 (11.80)	22.83 (4.77)	29.92 (5.47)
VII	3							10.31 (3.20)	34.87 (5.90)	66.32 (8.14)	100.44 (10.02)	131.23 (11.45)	36.30 (6.02)	24.47 (4.94)
VIII	7								13.55 (3.68)	22.62 (4.75)	42.11 (6.48)	61.82 (7.86)	41.27 (6.42)	57.19 (7.56)
IX	10									11.22 (3.34)	28.11 (5.30)	30.14 (5.49)	50.70 (7.12)	88.80 (9.42)
X	1										00.00 (00.00)	22.10 (4.70)	111.31 (10.55)	145.88 (12.07)
XI	2											14.68 (3.83)	121.49 (11.02)	173.82 (12.18)
XII	1												00.00 (00.00)	43.21 (6.57)
XIII	1													13.53 (3.67)

Values given in parentheses are $\sqrt{D^2}$ values; i.e., D.

Table 3. Cluster means for plant characters.

Cluster	Total no. of genotypes	Time to flowering (d)	Time from flowering to maturity (d)	Time to maturity (d)	Plant height (cm)	No. of branches	No. of pods/ plant	100-seed weight (g)	Biological yield (g)	Harvest index (%)	Seed yield/ plant (g)
I	1	68.00	46.00	114.00	46.30	3.50	82.00	2.60	23.40	31.45	6.90
II	10	70.20	45.65	112.90	43.35	2.40	62.90	2.30	15.43	20.36	3.17
III	2	68.00	50.75	118.75	44.75	2.25	59.00	1.70	11.00	28.65	3.15
IV	13	69.73	47.15	116.34	41.96	2.57	56.38	2.72	12.31	21.05	2.53
V	2	66.00	50.75	116.75	41.37	2.50	57.75	3.12	15.00	24.45	3.70
VI	3	64.66	41.33	106.00	42.60	2.33	65.50	2.35	12.13	28.05	3.45
VII	3	68.00	50.00	118.33	42.21	2.33	49.50	3.10	10.08	30.20	3.03
VIII	7	74.50	40.07	114.07	40.60	2.28	39.00	2.34	11.10	17.05	1.87
IX	10	76.75	37.90	114.75	43.08	2.70	69.20	2.03	14.65	19.19	3.12
X	1	84.00	33.00	117.00	40.75	2.00	39.50	3.05	16.00	18.60	3.05
XI	2	84.00	33.00	117.00	44.25	2.50	73.25	1.75	16.25	24.22	3.52
XII	1	64.00	47.50	116.50	45.55	2.50	80.50	2.10	18.00	22.20	4.00
XIII	1	64.00	54.00*	113.00	41.10	2.50	71.50	2.30	14.50	26.80	3.90

Table 4. Values of the first three canonical vectors and roots for lentil.

Vector	Time to flowering (d)	Time from flowering to maturity (d)	Time to maturity (d)	Plant height (cm)	No. of branches	No. of pods/ plant	100-seed weight (g)	Biological yield (g)	Harvest index (%)	Seed yield/ plant (g)	% of variation explained by canonical root	Value of canonical root
1	-0.4140	0.6898	0.0354	-0.1814	-0.2444	-0.2788	0.3059	-0.1183	0.1766	0.2057	52.92	0.00050
2	0.1456	-0.3444	0.5735	-0.2459	-0.2547	-0.2733	0.4264	0.1664	0.2217	-0.2691	14.09	0.00013
3	-0.3009	0.0104	0.3068	0.3691	-0.2278	0.0908	0.5269	0.2492	0.5134	-0.0124	8.60	0.00820

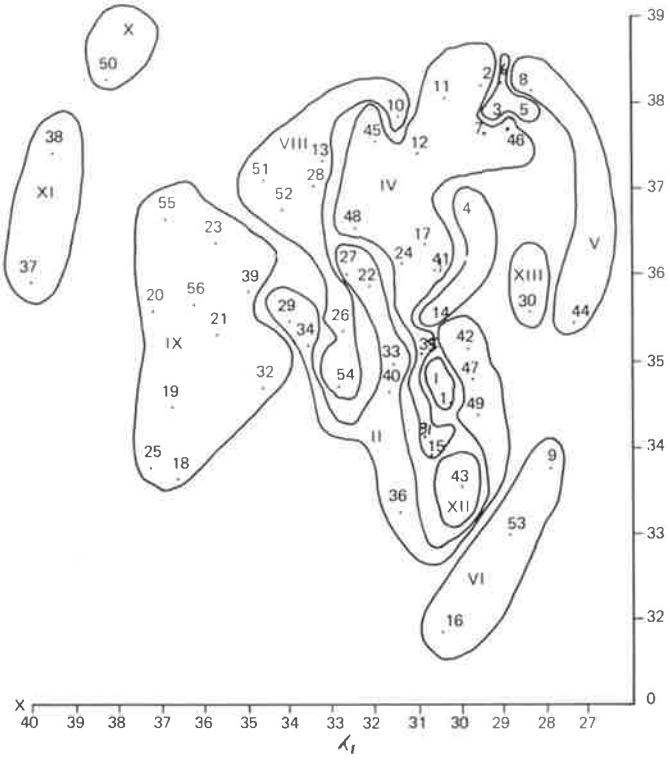

Fig. 2. Two-dimensional representation of divergence of lentil genotypes. The first three canonical vectors are co-ordinates, the clusters as obtained from D^2 analysis are super imposed.

The contribution of different characters to total genetic divergence was calculated; values of canonical vectors and roots are given in Table 4 and represented in Fig. 2. The first two canonical roots explained 67% of the genetic divergence. Of all characters, time from flowering to maturity contributed most to total genetic divergence (68.98%), followed by seed yield (20.57%) and harvest index (17.66%) in the first canonical root. Time from flowering to maturity and seed yield, accounting for 89.5% of the total genetic divergence, appeared to contribute most to genetic divergence in the lentil populations studied. Similar inferences were also drawn from the cluster means.

Genotypes from various regions occurred in the same cluster (see Table 2), showing that geographical diversity is not related to genetic diversity. Similar findings were reported by Tikka *et al.* (1977), Chandra *et al.* (1978), Singh (1982), and Sapra *et al.* (1984) while identifying parents for hybridization.

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Agronomy and Mechanization

Effect of fertilization, inoculation, and carbofuran on nodulation, yield, and protein content of lentil

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Abstract

A field experiment was conducted at the ICARDA sub-station, Terbol, Lebanon to determine the effect of phosphorus and nitrogen fertilization and *Rhizobium* inoculation on the nodulation, yield, and protein content of the locally adapted lentil cultivar "Syrian local large". The insecticide carbofuran was also used to reduce serious nodule damage caused by *Sitona* larvae and thus increase available nitrogen. The soil at Terbol proved to be highly fertile in major nutrient elements; phosphorus and nitrogen fertilizer application would be unnecessary, since it did not increase nodulation, yield, or protein content. Inoculation with *Rhizobium* was also found unnecessary because the local rhizobial population seemed to be efficient and sufficient for nodulation. Carbofuran did not increase yield, but it did increase (1) nodule mass by significantly reducing *Sitona* nodule damage, and (2) seed protein content slightly, which increased protein yield/hectare.

Introduction

Lentil is an important source of protein in many developing countries. The average yield is low, however, and has remained almost static during the last few years (FAO 1982), probably because of improper fertilization, inoculation, and pest management.

Lentils, which are rich in protein, may require about 100 kg N and 28 kg P_2O_5/ha to produce 2 tons of seed/ha (Saxena 1981). Both P and starter N, required particularly in soil of low fertility, are important to enhance N_2-fixation. A major portion of the N requirement may be met by symbiotic N_2-fixation, while P is provided exclusively from the soil. The response of lentils to N and P fertilization as well as to *Rhizobium* inoculation varies in different locations due to differences in climatic, edaphic, and biotic factors. However, in a favorable environment, symbiotic N_2-fixation may be limited by the lack of effective *Rhizobium* strains and/or serious nodule damage by larvae of *Sitona* weevil—the situation in many lentil growing countries of North Africa and West Asia (Islam 1982). In northern Syria, this insect causes up to 97% damage to lentil root nodules (Tahhan and Hariri 1982). Carbofuran, a soil-applied insecticide, was found effective in reducing this damage by more than 70% (Islam and Afandi 1982; Itani *et al.* 1985). Our experiment was therefore designed to determine the effect of N and P fertilization, *Rhizobium* inoculation, and carbofuran on nodulation, yield, and protein content of lentils.

Materials and Methods

A field experiment was conducted at the ICARDA sub-station at Terbol in the Beqa'a valley of Lebanon during the 1984/85 winter season using a locally adapted lentil cultivar "Syrian local large" (ILL 4400). The experiment was laid out in a randomized complete block design with four replications. It tested eight treatments as combinations of 50 kg P_2O_5, 50 kg N, 1 kg a.i. carbofuran/ha, and inoculation. The treatments were: (1) control, (2) P, (3) carbofuran, (4) inoculation, (5) inoculation plus P, (6) inoculation plus carbofuran, (7) inoculation, P, and carbofuran, and (8) N, P, and carbofuran. Preplanting N and P were applied as urea and triple superphosphate, respectively, and carbofuran was used as furadan 5G. Inoculum was obtained from the microbiology laboratory of ICARDA, Aleppo, Syria. To avoid any interaction, no other pesticides were used, and plots were kept weed-free by hand weeding.

Representative soil samples from the control plots were analyzed following Black *et al.* (1965) to determine some physical and chemical characteristics, which are presented in Table 1 along with the methods of analysis.

Table 1. Soil characteristics at the experimental site.

Characteristic	Value	Method employed
Soil reaction (pH)	7.23	1:2.5 soil-to-water suspension by glass electrode pH meter
Soil texture (%)		
Sand	26.2	Hydrometer method
Silt	12.0	
Clay	61.8	
Organic matter (%)	1.62	Walkely-Black method
Primary nutrients (ppm)		
Total N	1280.0	Macro-Kjeldahl method
Available P	56.5	$NaHCO_3$ extraction method
Exchangeable K	631.3	CH_3COONH_4 extraction method

The numbers of healthy and damaged nodules were counted from 10 representative plants on 30 Jan (seedling stage), 2 Apr (pre-flowering), and 18 Apr (50% flowering). The weight of healthy nodules was determined after drying at 70°C for 48 hours in an air-draft oven. Another 10 randomly-selected plants were used to determine primary yield components. Protein content of seeds was measured using a near-infrared analyzer (Neotec^R, Model 51A, and that of straw by the macro-Kjeldahl method (Horwitz 1975). The analyzer was standardized with the second method. Crude protein % was calculated by multiplying the N content by 6.25. Analysis of variance was computed for the randomized complete block design, and Duncan's Multiple Range Test (DMRT) was used for comparison among treatment means. Correlation and regression of nodulation with yield and protein content were also computed.

Results and Discussion

Nodulation

The numbers of healthy nodules, and those damaged by *Sitona* larvae, were recorded on three sampling dates (Table 2). There were no damaged nodules on the first sampling date (30 Jan), since *Sitona* weevils are not active during cold weather (Hariri 1979). These observations concur with those previously reported by Islam and Afandi (1982). However, considerable nodule damage was observed on the subsequent sampling dates (2 and 18 Apr). Because of its insecticidal activity, carbofuran reduced nodule damage and thus, increased nodule mass (Table 3). Similar results were reported for lentils by Islam and Afandi (1982) in Syria. This increased nodule mass may lead to increased N_2-fixation and ultimately affect yield and protein content.

The application of P did not affect nodulation, probably because available P in native soil (56.5 ppm) was several times higher than the critical level (4 ppm according to Matar 1976). In addition, the neutral soil reaction (pH 7.23) and adequate moisture (425 mm rain during growing season) created favorable soil conditions for P uptake. Similarly, N fertilization had no effect, perhaps due to the naturally high N level (1280 ppm) in the soil. The ineffectiveness of inoculation indicated that the native rhizobial populations were as efficient in N_2-fixation and as competitive as the introduced strain. This conclusion was supported by the good nodulation observed in the control plots. These results conform with those reported in India by Saxena and Rana (1977) using a local cultivar.

Grain yield and yield components

Grain yield was not affected significantly by any of the treatments (Table 4). These treatments had no effect on seed yield/plant, although differences in yield components were statistically significant. This was due to yield component compensation (Adams

Table 2. Mean number of healthy and damaged nodules/plant as affected by fertilization, inoculation, and carbofuran on three sampling dates.

	Healthy nodules				Damaged nodules[1]		
Treatment	**30 Jan**	**2 Apr**	**18 Apr**	**Mean**	**2 Apr**	**18 Apr**	**Mean**
Control	14.0ab	17.7bc	4.5a	12.1	8.5a	11.4abc	10.0
Phosphorus (50kg P_2O_5/ha)	12.7ab	17.2bc	4.5a	11.5	9.0a	12.0abc	10.5
Carbofuran (1kg a.i./ha)	13.0a	29.7ab	7.9a	16.9	4.4b	9.5bc	7.0
Inoculation	12.8ab	13.2c	5.6a	10.5	9.1a	16.5a	12.8
Inoculation and phosphorus[2]	12.4b	17.9bc	4.1a	11.5	0.6a	16.6a	13.6
Inoculation and carbofuran[2]	16.0a	32.7a	7.9a	18.9	3.9b	13.2ab	8.6
Inoculation, phosphorus, and carbofuran[2]	12.4b	28.8b	8.2a	16.5	4.1b	7.6c	5.9
Nitrogen (50kg N/ha), phosphorus[2], and carbofuran[2]	11.9b	28.3ab	8.8a	16.3	5.3b	12.5abc	8.9
Mean	13.0	23.3	6.4	14.3	8.9	12.4	9.7

Values within a column followed by the same letter are not significantly different at 5% probability level according to DMRT.

[1] There were no damaged nodules on the first sampling date.

[2] Phosphorus and carbofuran rates are the same as those used in the second and third treatments, respectively.

Table 3. Weight of healthy nodules/plant as affected by fertilization, inoculation, and carbofuran at three sampling dates.

	Nodule weight (mg/plant)			
Treatment	**30 Jan**	**2 Apr**	**18 Apr**	**Mean**
Control	4.0a	5.3b	0.9a	3.4
Phosphorus (50kg P_20_5/ha)	4.5a	5.2b	0.9a	3.5
Carbofuran (1kg a.i./ha)	4.7a	9.2a	2.0a	5.3
Inoculation	4.7a	5.1b	1.3a	3.7
Inoculation and phosphorus*	4.3a	4.9b	0.9a	3.4
Inoculation and carbofuran*	4.8a	10.8a	1.7a	5.8
Inoculation, phosphorus*, and carbofuran*	4.1a	9.4a	1.8a	5.1
Nitrogen (50kg N/ha), phosphorus*, and carbofuran*	3.8a	9.5a	1.9a	5.1
Mean	4.4	7.4	1.4	4.4

Values within a column followed by the same letter are not significantly different at 5% probability level according to DMRT.

*Phosphorus and carbofuran rates are the same as those used in the second and third treatments, respectively.

Table 4. Yield, yield components, and protein content of lentil as affected by fertilization, inoculation, and carbofuran.

Treatment	Yield/ plant (g)	Pods/ plant (no.)	Seeds/ pod (no.)	100-seed weight (g)	Grain yield (t/ha)	Grain protein (%)	Straw protein (%)	Protein yield (kg/ha)
Control	1.29a	22.9abc	1.13ab	5.9a	2.12a	24.2abc	8.0a	832ab
phosphorus (50kg P_2O_5/ha)	1.35a	23.3abc	1.23ab	5.8a	2.08a	24.0bc	8.1a	832ab
Carbofuran (1.0kg a.i./ha)	1.32a	21.9bc	1.20ab	5.9a	2.17a	24.7abc	8.4a	899a
Inoculation	1.73a	25.6ab	1.20ab	5.8a	2.01a	24.0bc	8.3a	817ab
Inoculation and phosphorus*	1.53a	27.2a	1.28a	5.7a	1.95a	23.6c	8.2a	787b
Inoculation and carbofuran*	1.33a	22.8abc	1.13ab	5.9a	2.10a	25.0a	8.6a	906a
Inoculation, phosphorus*, and carbofuran*	1.34a	20.9c	1.23ab	5.8a	2.03a	24.6a	8.0a	811a
Nitrogen (50kg N/ha), phosphorus*, and carbofuran*	1.30a	22.1bc	1.10b	5.8a	1.89a	24.3a	9.2a	849a
Mean	1.40	23.3	1.18	5.8	2.05	24.3	8.4	843

Values within a column followed by the same letter are not significantly different at 5% probability level according to DMRT.
*Phosphorus and carbofuran rates are the same as those used in the second and third treatments, respectively.

1967) whereby a high value in one component is compensated for by a low value in other component(s). For example, inoculation along with P gave the highest number of pods/plant and seeds/pod, but the lowest seed weight. The lack of response in grain yield to N, P, and inoculation can also be attributed to high soil fertility and the presence of effective strains of native *Rhizobium leguminosarum*. Although carbofuran increased nodule mass, it did not increase yield. The increased nodule mass due to carbofuran may not necessarily improve yield in a high fertility soil like that of the experimental site, since the energy requirement for N_2-fixation may burden the plant when N is readily available from the soil at a lower energy cost. In spite of the improved nodulation, grain yield was not increased, because more photosynthates are required to support nodules. This was also evident from the negative correlation coefficients between grain yield and nodule mass (Table 5).

Protein content

Seed protein content and protein yield differed significantly among treatment means (Table 4). Fertilization and inoculation did not affect protein content. Carbofuran alone or in combination with inoculation increased grain protein content and thus protein yield. This was perhaps due to the reduced nodule damage by carbofuran. A positive association of the number and weight of healthy nodules with the protein content of grain and straw supports this view (Table 5). Most probably, more N was fixed in carbofuran-treated plots, because of higher nodule mass, which contributed to greater N availability to the seed and straw, thus resulting in increased protein levels. The contribution of higher nodule mass in carbofuran-treated plots, however, depended on the growth stage, and the amount of N fixed at the flowering stage contributed more, as indicated by the higher slope of the regression line at that stage (Fig. 1).

Table 5. Simple correlation coefficient between characteristics at the second and third sampling dates.

Character	Number of healthy nodules		Number of damaged nodules		Weight of healthy nodules	
	2 Apr	18 Apr	2 Apr	18 Apr	2 Apr	18 Apr
Grain yield (t/ha)	-0.16	0.32	0.04	-0.11	-0.32	-0.18
Biological yield (t/ha)	0.26	0.34	0.05	-0.16	0.26	0.36*
Grain protein (%)	0.42*	0.39*	-0.23	-0.28	0.48**	0.41*
Straw protein (%)	0.30	0.35*	-0.09	-0.09	0.44*	0.44*

*Significant at 5% probability level.

**Significant at 1% probability level.

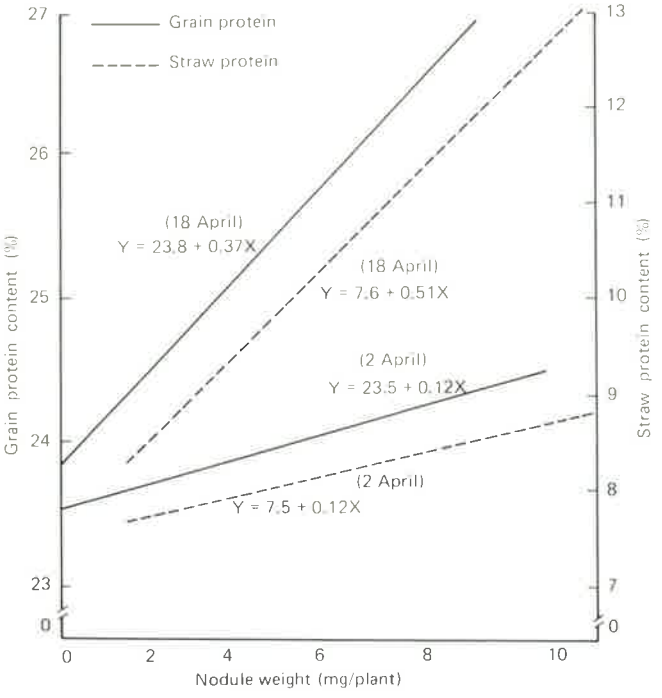

Fig. 1. Relationships of grain and straw protein content with nodule weight/plant at the second and third sampling dates, 1985.

In conclusion, the results indicated that the soil at the Terbol Station was fertile enough in N and P not to require artificial fertilization for lentil. The native rhizobial population was sufficient and efficient enough to permit adequate N_2-fixation, so that inoculation was unnecessary. Carbofuran, however, was effective in reducing nodule damage from *Sitona* weevil and improving protein content in seed and straw. Because of the high soil fertility, however, grain yield did not increase. It would be interesting to carry out such an experiment in soil of low fertility.

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Physiology and Microbiology

Distribution of empty pods in lentils

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Abstract

The frequency and nodal position of empty pods was studied at three plant population levels (40, 110, and 220 plants/m^2) in the 1983/84 season in Chile. At the first nodal position on primary branches, there was an average of 10.1% empty pods, but by the sixth node, 83% of pods were seedless. The acropetal, increasing frequency of empty pods was confirmed on secondary branches. Increased plant competition also increased the frequency of barren pods.

Introduction

Araucana INIA, a large seeded cultivar released by INIA (Instituto de Investigaciones Agropecuarias), is now being grown by farmers in Chile. It has good yield potential and adaptability to lentil growing areas in Chile. When sown at the normal sowing date, however, the plant produces an average of 25 to 45% empty pods, depending on location and growing season. This cultivar has a higher yield potential than has been realized; although it yields as much as 3,480 kg/ha, this level was reached when 33% of the pods were empty (Penaloza and Mera 1984). So reducing the number of empty pods should further increase yields. With this in view, trials were conducted by Food Legume Program at Estacion Experimental Carillanca to study the factors contributing to empty pods in lentil. This paper investigates the location of empty pods under different levels of plant competition.

Materials and Methods

An experiment on plant density was carried out at the Estacion Experimental Carillanca, Chile, during the 1983/84 season. Plants from three plant population treatments (40, 110, and 220 plants/m^2) were evaluated for empty pod character. The treatments were grown in a randomized block design with four replications. The plots were 4 m long with five rows 34 cm apart. At harvest, 10 plants were selected from the central row and put in separate paper bags. Primary and secondary branches were examined separately for full and empty pods at each nodal position on a branch. Since the number of reproductive nodes/branch decreased as plant density increased, only the nodes common to both types of branches were included in the statistical analysis. The analysis followed a split-plot design, with plant density as main plot and node position as sub-plot for primary and secondary branches, respectively.

Results and Discussion

At each plant density, the percentage of empty pods on both branch types increased from the basal to the apical nodes (Table 1). This pattern was related to the variation in seed weight within the canopy (unpublished work). A decline in weight/seed acropetally was reported in chickpea (Sheldrake and Saxena 1976), suggesting that pod filling was limited by the supply of assimilates or other nutrients.

Table 1. Percentage of empty pods at successive pod-bearing nodes at three plant densities on (a) primary and (b) secondary branches.

(a) Primary branches

Nodal position	Density (plants/m^2)			Mean	S.E. mean
	40	110	220		
1	10.5	10.5	9.3	10.1	
2	7.5	10.6	17.7	12.0	
3	20.6	26.8	40.4	29.2	±3.76
4	23.4	35.6	57.0	38.7	
5	57.2	51.4	63.6	57.4	
6	74.5	83.1	92.5	83.4	
Mean	32.3	36.3	46.8		
S.E. mean		±2.81			

(b) Secondary branches

Nodal position	Density (plants/m^2)			Mean	S.E. mean
	40	110	220		
1	15.9	26.3	31.6	24.6	
2	27.7	47.2	65.3	46.7	±2.19
3	53.9	79.4	83.1	72.4	
Mean	32.5	51.0	60.3		
S.E. mean		±5.35			

Therefore, it is expected that the high proportion of unfilled pods in the upper part of the plant canopy could be due to the lack of assimilates to young pods developing during the later stage of the reproductive phase. That is probably because of the declining leaf area as plant senescence advances (Pandey 1983). Environmental conditions such as a wet period during flowering (Miroshnichenko and Kolotilov 1978) or cloudy weather (Aziz, Khan, and Shah 1960) have also been found to have adverse effects on the seed set in legumes and could be responsible for the lack of pod filling.

At each nodal position on a branch, the percentage of empty pods increased as plant density increased (Table 1). This trend was again similar to that of seed weight. An increase in mutual shading at higher density during the flowering period probably reduced the supply of assimilates to reproductive sinks, resulting in a high proportion of unfilled pods.

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Pests and Diseases

The effect of successive infestation with bruchid beetles on some physical properties and chemical constituents of stored lentils

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Abstract

In this study of lentil infestation by three bruchid species, lentil seeds were most infested by *Bruchidius incarnatus*, followed by *Callosobruchus chinensis*, and then by *C. maculatus*. The three bruchids reduced the seed weight and also inhibited seed germination, following the same ranking of species. Increased infestation was accompanied by increased moisture in infested seeds. The increased moisture content had reached similar levels for the three species by the end of the experiment.

As far as the bruchids' effect on the chemical constituents of lentil seeds, *B. incarnatus* caused the greatest losses in carbohydrates and fatty acids, followed by *C. chinensis*, while *C. maculatus* caused the lowest losses. *C. chinensis* produced the greatest reduction in protein content, followed by *C. maculatus* and *B. incarnatus*. The two *Callosobruchus* species produced a similar reduction in free amino acid content, while *B. incarnatus* produced the least such reduction.

Introduction

Lentils are widely grown in Egypt in order to meet domestic needs. Lentils are harvested in the spring, but consumed throughout the year, particularly in winter. Accordingly, seeds must be stored and protected against storage pests.

Seed stores furnish a suitable habitat for insects to multiply until food reserves are exhausted. Pre-harvest infestation is very serious because weevil populations can multiply dramatically during storage; within six to eight months, they may damage 80% of the seeds (Caswells 1961).

This study was carried out to assess the capability of three bruchids (*C. maculatus, C. chinensis*, and *Bruchidius incarnatus*) to survive and develop for three generations on stored lentils. The effects of insects on seed weight loss, moisture content, germination percentage, and chemical constituents were also investigated.

Materials and Methods

Lentil seeds were fumigated with methyl bromide and then aerated to remove fumigant residues. Six groups of 5 kgs seeds each were incubated in jute bags for two weeks at 30 +1°C and 75% RH. The Buhler rapid moisture tester was used to estimate the moisture content of seeds at the beginning of the experiment (10.3%). The infestation of stored seeds with the three bruchid beetles was assessed according to the method adapted by Nakamura (1962).

The emerged adults of each species were transferred to the lentils in the jute bags, with a hundred pairs (female, male)/kg seeds.

The infested bags were then tightly closed and kept under identical conditions. Adults were allowed to oviposit for only one week and were then removed. The egg-infested seeds were kept for the development of the progeny. The same procedure was followed with the second and third insect generations. Treatments were replicated twice.

After removing the emerged adults of each generation, infestation rates were estimated using 500 seeds of each replicate. The infested seeds were counted as the rate of external infestation, and the remainder of the sample seeds examined were dissected with a scalpel to estimate the rate of internal infestation. The influence of infestation on weight loss, moisture content, germination percentage, and seed constituents were investigated. The methods used are summarized below.

a) Determination of weight loss: Five-hundred infested and sound seeds were sieved and weighed. The weights were converted to dry weights (D.W.), and the percentage of weight loss was calculated with the following equation:

$$\% \text{ weight loss} = 100(\text{D.W. sound seed} - \text{D.W. infested seed}).\ (\text{D.W. sound seed})^{-1}$$

Table 1. Infestation percentages and their standard errors of lentils by bruchid beetles during three generations.

Generation	Infestation (%)								
	C. maculatus			*C. chinensis*			*B. incarnatus*		
	Internal	External	Total	Internal	External	Total	Internal	External	Total
1	0.4	6.4	6.8	1.0	11.5	12.5	1.0	20.2	21.2
2	1.0	19.2	20.2	2.0	22.8	25.8	1.5	35.6	37.1
3	2.1	31.5	33.6	1.8	42.3	44.1	1.0	62.5	63.5
Mean			20.2 ± 5.24			27.3 ± 6.0			40.6 ± 7.86

b) Determination of moisture content: Samples of 10 g were taken from sound and infested seeds to determine moisture content.

c) Determination of germination percentage: The seed germination percentage was estimated from 100 seeds germinated for two weeks.

d) Determination of seed chemical constituents: Samples of sound and infested seeds were ground separately, dried, and analyzed for total carbohydrate content. This was measured in the form of reducing sugars, following the Shaffer-Hartman method (1921), as modified by Maskell and El-Gawadi (1936), with the data expressed as simple sugars. Also analyzed were the contents of: total nitrogen, using the Kjedahl method (AACC 1962); total free amino acids, using the method described by Abdel-Hafez *et al.* (1977); and total fatty acids, using the method described by Joseph *et al.* (1972).

Results and Discussion

Incidence of infestation

The data in Table 1 indicate the significant effect of bruchid species on the infestation percentage of lentil seeds, as weil as on the duration of insect attack. The results show the highest infestation by *B. incarnatus*, (40.6%), followed by *C. chinensis* (27.5%), and *C. maculatus* (20.2%).

Infestation increased with each generation of insects, resulting in a serious and cumulative loss of seeds. These findings are similar to those of El-Bamby *et al.* (1970), who reported that lentils were the food most favored by *B. incarnatus*, allowing the fastest development of the insect after hatching and producing the highest emergence percentage, the heaviest and longest-lived beetles, and the most prolific females. Koura *et al.* (1971) also reported that the order of decreasing suitability of *C. maculatus* to various plants was as follows: cowpeas > beans > soybeans > lentils.

Relationship between seed properties and bruchid infestation

Table 2 shows the influence of bruchid infestation of lentils on seed properties: weight, moisture content, and germination. The significant reductions in weight and germination percentage were due to insect infestation. The particular species of bruchid and the succession of attack has the greatest influence on these percentages. By the end of the experiment, *B. incarnatus* caused the highest weight loss in stored seeds (64.1%), followed by *C. chinensis* (53.5%), and then by *C. maculatus* (33.3%). As infestation level increased with successive generations, so did weight loss.

The mean loss in infested lentil seeds was 42.2% for *B. incarnatus*, 34.9% for *C. chinensis*, and 20.7% for *C. maculatus* within the three successive rounds

Table 2. Effect of infestation by bruchid beetles on weight loss, moisture content, and germination of lentil seeds and their correlations with germination percentage.

Insect species	Weight of 500-dried seeds (g)	Weight loss (%)	Moisture content (%)	Change in moisture content	Germination (%)
C. maculatus	11.8	-	10.3	-	96
	9.9	12.3	11.1	+0.8	71
	9.5	16.6	11.7	+1.4	30
	7.8	33.3	12.8	+2.5	23
	r=0.946		r=+0.970	r=-0.951	
	b=+0.783		b=+0.070	b=-2.210	
C. chinensis	11.8	-	10.3	-	96
	9.5	16.2	11.3	+1.0	65
	7.4	34.9	12.0	+1.7	31
	5.4	53.5	12.9	+2.6	12
	r=+0.996		r=+0.858	r=0.975	
	b=+1.170		b=+0.050	b=1.920	
B. incarnatus	11.8	-	10.3	-	96
	9.0	20.6	11.6	+1.3	68
	6.6	41.8	12.8	+2.5	25
	4.1	64.1	13.3	+3.0	7
	r=+0.994		r=+0.951	r=-0.965	
	b=+1.010		b=+0.048	b=-1.467	
L.S.D. (5%)	Beetles 0.74		0.22	3.4	
	Generations 0.86		0.26	3.9	

of infestation. The coefficients of correlation with percentage germination were strong and positive (+0.994, +0.996, and +0.783), while the regression coefficients were +1.010, +1.170, and +0.783 for the three beetles, reported in the same order as above. This is similar to the results of El-Sawaf (1956), El-Bamby *et al.* (1970), and Koura *et al.* (1971).

Infestation also reduced germination percentage and, hence, seed viability. The correlation coefficients (-0.965 for *B. incarnatus*, -0.975 for *C. chinensis*, and -0.951 for *C. maculatus*) indicated a negative relationship between insect infestation and viability. By the third generation of infestation, seeds had almost lost their viability. This was more pronounced in seeds infested with *B. incarnatus* (7% germination), followed by *C. chinensis* (12%) and *C. maculatus* (23%). A significant reduction in germination percentage occurred after the first infestation, with viability losses of 29.1%, 32.2% and 26.0% compared to sound seeds. After two rounds of infestation, loss rates reached 73.9% in *B. incarnatus*, 67.7% in *C. chinensis*, and 68.7% in *C. maculatus*.

Table 3. Effect of infestation by bruchid beetles on some chemical constituents of lentil seeds.

Insect	Carbohydrate		Protein		Free amino acids		Fatty acids	
	Total (mg/g)	Loss (%)	Total (mg/g)	Loss (%)	Total (mg/g)	Loss (%)	Total (mg/g)	Loss (%)
C. maculatus	253.5	-	318.0	-	0.6249	-	10.04	-
	235.0	7.3	274.0	13.8	0.6057	3.1	8.92	11.2
	197.9	21.9	268.9	15.4	0.5865	6.1	8.18	18.5
	167.0	34.1	264.1	16.9	0.4904	21.5	7.29	27.4
Mean		21.1		15.4		10.2		19.0
C. chinensis	253.5	-	318.0	-	0.6249	-	10.04	-
	228.8	9.7	273.5	14.0	0.6057	3.1	7.29	27.4
	197.9	21.9	268.2	15.7	0.5440	12.9	6.84	31.9
	142.2	43.9	232.1	27.0	0.5330	14.7	6.09	39.3
Mean		25.2		18.9		10.2		32.9
B. incarnatus	253.5	-	318.0	-	0.6249	-	10.04	-
	228.7	9.8	310.3	2.4	0.6200	0.8	7.29	27.4
	191.7	24.4	294.1	7.5	0.6000	4.0	6.20	38.2
	129.8	48.8	274.2	13.8	0.5330	14.7	5.80	42.2
Mean		27.6		7.9		6.5		36.0
L.S.D. (5%) Beetles =	n.s.[1]	9.312		n.s.			0.451	
Generations =	22.494	10.753		0.0274			0.521	

[1] n.s. = non significant.

The adverse effects of insect infestation on the viability of stored seeds were reported by Gujar (1976). In that study, infestation by *C. chinensis* and *C. maculatus* caused a significant reduction in germination, especially during the first 9-12 days after oviposition. Southgate (1979) reported that germination percentage was correlated with the number of emergence holes in the seed, the seedling growth rate, and seedling vigor; that is, the more holes, the slower the germination and the greater reduction in seedling vigor.

The data show that the increase in infestation was accompanied by a moisture increase in the infested seeds. The moisture content of uninfested seeds increased from 10.3 to 13.3% with *B. incarnatus*, to 12.9% with *C. chinensis*, and to 12.8% with *C. maculatus*. Also, the increased moisture content of lentils due to infestation with the three insect species was almost the same in each case by the end of the experiment, in conformation with the results of Altahtawy *et al.* (1975).

Effect on some chemical constituents of lentil seeds

Data in Table 3 indicate that the increased infestation by any of the three bruchid beetles markedly decreased the contents of carbohydrate, protein, free amino acids, and fatty acids in lentil seeds. Sound seeds had an average carbohydrate content of 25.4%, while carbohydrate losses were 21.2, 25.2, and 27.6% with infestation by *C. maculatus*, *C. chinensis*, and *B. incarnatus*, respectively. The carbohydrate losses were related to the level of infestation by the three species.

Protein loss due to bruchid infestation differed significantly according to bruchid species: 13.8% and 16.9% loss from *C. maculatus*, 14.0% and 27.0% from *C. chinensis*, and 2.4% and 13.8% for *B. incarnatus* in the first and third rounds of infestation. *C. chinensis* caused the greatest reduction in protein content, followed by *C. maculatus* and then by *B. incarnatus*.

Table 3 shows that the free amino acid (FAA) loss due to bruchid infestation was correlated significantly with infestation level. Loss in FAA content reached 3.1%, 6.1%, and 21.5% at the three levels of infestation by *C. maculatus*. The two *Callosobruchus* species produced a similar reduction in FAA content of lentil seeds (10.2% in both cases), while *B. incarnatus* produced the lowest reduction (6.5%).

B. incarnatus reduced fatty acid content the most of any species, and *C. maculatus* the least. The percent loss of fatty acids varied between 11.2-27.4 for *C. chinensis*, 27.4-39.3 for *B. incarnatus*, and 27.4-42.2 for *C. maculatus*, all in the first and third rounds of infestation.

In conclusion, the infestation with the three bruchid species slightly decreased the amounts of some chemical constituents of seeds after the first generation; loss rates of food components increased as infestation progressed (second and third generation). This concurs with the results of Gujar (1976), who found that the initial feeding period of 12 days in *C. maculatus* and 9 days in *C. chinensis* was characterized by very slow feeding. The next period (lasting 27 days in the first species and 22 days in the second) was characterized by very rapid feeding.

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Seed coat darkening in lentil

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Abstract

In lentil, the rate of seed coat darkening is affected by storage conditions. In this study, it was found that pre-harvesting environmental conditions also affected the rate of darkening.

Introduction

During storage, lentil seed coats may turn dark brown, rendering the seeds unacceptable to the consumer. The darkening process is greatly influenced by storage conditions (de Bezeda 1980). Seed coats may darken within a week under high temperatures and humidities as well as in the presence of light. This study sought to determine the effect of pre-harvesting environmental conditions on the rate of seed coat darkening.

Materials and Methods

Seeds of Eston, Laird, Redchief, and P.I.345635 were grown at four locations with two replications. After harvesting, seed samples from each plot were frozen until analysis. Samples from each plot were divided into four groups of 25 ml each and placed in petri dishes. Darkening was accelerated by placing the petri dishes in a growth chamber at 38°C, 30% RH, with a light quantum of 402 micromol m^{-2} sec^{-1}. Seed coat darkening was measured with an Agtron M-50-A at 546 nm before storage and after 2,4,6,8,10,12,14,17,21, and 51 days in the growth chamber. The rate of seed coat darkening was estimated by regressing seed coat color (absorbance) on duration of storage. The slope of the regression line (b value) is the rate of seed coat darkening in relative absorbance/hour. All linear regressions were significant and had a high coefficient of determination (See Table 1).

Table 1. Rate of lentil seed coat darkening as determined by linear regression of seed coat color (relative absorbance) over hours of storage for five lentil lines, 1983.

Location and cultivar	b ± s.e.	r^2
Elrose		
Redchief	2.47×10^{-4} ($\pm$ 0.69×10^{-5})	98.5
Laird	2.55×10^{-4} ($\pm$ 1.34×10^{-5})	95.0
Eston	2.67×10^{-4} ($\pm$ 1.00×10^{-5})	97.4
Goodale		
Redchief	2.43×10^{-4} ($\pm$ 1.04×10^{-5})	96.6
Laird	2.66×10^{-4} ($\pm$ 1.56×10^{-5})	93.6
Eston	2.75×10^{-4} ($\pm$ 1.27×10^{-5})	96.1
Hagen		
Redchief	3.54×10^{-4} ($\pm$ 1.93×10^{-5})	94.6
Laird	2.82×10^{-4} ($\pm$ 1.59×10^{-5})	94.2
Eston	3.42×10^{-4} ($\pm$ 1.51×10^{-5})	96.4
Indian Head		
Redchief	3.59×10^{-4} ($\pm$ 1.86×10^{-5})	95.1
Laird	3.73×10^{-4} ($\pm$ 2.10×10^{-5})	94.3
Eston	3.60×10^{-4} ($\pm$ 1.97×10^{-5})	94.6
Saskatoon		
P.I. 345635	-1.23×10^{-4} ($\pm$ 0.74×10^{-5})	93.6

Results and Discussion

The seed coats of P.I. 345635 did not darken with time, but lightened (negative regression) due to bleaching. Therefore, P.I. 345635 was excluded from

Table 2. Analysis of variance for rate of seed coat darkening[+] of three lentil cultivars grown at four locations, 1983.

Source of variation	D.F.	MS	F
Locations	3	1631 x 10^{-11}	245.0**
Replicates in locations	4	5.2 x 10^{-11}	0.78^{ns}
Cultivars	2	60.5 x 10^{-11}	9.10**
Location x cultivar	6	110.0 x 10^{-11}	16.5**
Error	8	6.64 x 10^{-11}	
Total	23		

[+] Relative absorbance.

**Significant at P = 0.01.

the analysis of variance. This line is characterized by a lack of proanthocyanidin and other flavonoids (Vaillancourt and Slinkard 1983), which probably explains the lack of darkening. The rates of seed coat darkening can be divided into two groups: one with a slow rate of darkening, corresponding to the Elrose and Goodale sites; and one with a more rapid rate of darkening, corresponding to the Indian Head and Hagen sites. Individual plot data on the rate of seed darkening (b value) were analyzed statistically (Table 2). The analysis of variance showed that locations and cultivars significantly influenced the rate of darkening, but that locations explained a greater fraction of the total variance than did cultivars. The interaction between cultivar and location was significant, probably because Laird at Hagen (Table 1) had a much lower rate of darkening than the other cultivars at that location.

Pre-harvesting environmental conditions largely determine the rate of lentil seed coat darkening. The significant location effects probably resulted from rain on mature plants in the field at some locations, and little or no rain at other locations, since rain-dampened seed coats will subsequently darken faster than seed coats that were never dampened. Thus, the reason that the seed coats of Laird at Hagen darkened more slowly than the other lines at the site (Table 1) was probably that Laird matures later than the other cultivars, and its mature seed may not have received as much rainfall before harvest. Thus, seed coats of Laird would be expected to have a lower rate of darkening over a given period of time.

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de Bezeda, M.V. 1980. Effect of environmental conditions on lentil seeds. M.Sc. thesis. University of Ottawa, Ottawa.

Vaillancourt, R. and Slinkard, A.E. 1983. Variation in tannin content of lentils. LENS 10(1): 36.

LENTIL INFORMATION

LENS Bookshelf

Summerfield, R.J. and Roberts, E.H. (eds.) 1985. **Grain Legume Crops**. Collins Professional and Technical Books, Dept. 44, 8 Grafton St., London W1 X 3LA, U.K. 880pp. ISBN 0-00-383037, £40.

Grain legumes such as soybeans, groundnuts, and common beans are important world crops. Others such as cowpeas, lentils, and mung beans are regionally important, especially in warmer areas, and are rapidly increasing in economic significance. There is considerable scope for improving and stabilizing the yields of these crops and international research on them has expanded dramatically over the past few years.

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- An appendix on recent trends in internationally-oriented grain legume research.

Agriculture Canada. Canadian Pulses Report

This report, published several times a year by the Marketing and Economics Branch of Agriculture Canada, disseminates information on marketing and economics. Each issue summarizes the Canadian and U.S. monthly pulse market trends and monthly offering pulse price range, U.S. monthly pulse dealer selling price range, and U.S. pulse grower price range; reports on the world pulse market by countries of destination and countries of origin; and gives foreign agriculture reports. To receive this report regularly, write:

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Key Lentil Abstracts

Beauchamp, C.J. 1985. **Effects of foliar-applied fungicides of ascochyta blight of lentil.** *M.Sc. thesis.* University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO Canada.

The effect of different fungicides on *Ascochyta lentis* was investigated in laboratory and field tests. Laboratory tests were conducted to determine the fungi toxicity of 16 fungicides on conidial germination and mycelial growth. Captafol, chlorothalonil, folpet, metiram, mancozeb, and dodine inhibited conidial germination at less than 100 µg/ml. Benomyl, DPX H6573, thiophanate, propiconazole, dinocap, captafol, and mancozeb gave 95% inhibition of mycelial growth at less than 150 µg/ml. In field tests, the effects of foliar-applied fungicides on seed yield and frequency of infection were studied in 1983 and 1984 in artificially inoculated plots. In one set of experiments, 11 fungicides were evaluated using a single application at early bloom to early pod set with Common Chilean lentil (*Lens culinaris* Medic.), a susceptible type. In general, systemic fungicides showed poor potential to increase seed yield, whereas protectant fungicides were more promising. Captafol (1.2 kg a.i./ha) and chlorothalonil (1.7 kg a.i./ha) seemed to be the best prospects. In a second set of experiments, different spraying schedules were tested using eight combinations of three dates. In 1983, benomyl (1.0 kg a.i./ha) and captafol (1.2 kg a.i./ha) were applied, while in 1984 captafol (1.2 kg a.i./ha) and chlorothalonil (0.85 and 1.7 kg a.i./ha) were applied at nine ($\pm$ 1) day intervals. In the first summer, captafol gave mean seed yield increases of about 30% and mean decreases in frequency of seed infection of 25% compared to their checks. The effects of benomyl were slight. Also, seed yield increased by 15% with two or three sprays of captafol compared to one spray. The frequency of seed infection showed the same tendency with a decrease of about 20%. In any treatments involving the first date of application, seed yield increased by about 25% and frequency of seed infection decreased by 30% compared to applications carried out nine or 18 days later. The first date of applying an effective fungicide is as important as the number of applications to give seed yield increases and decreases in frequency of seed infection. In 1984, dry, hot weather inhibited disease development and no conclusion could be drawn from the results. In the third set of experiments involving four combinations of two dates of application with benomyl and captafol in 1983, there were only slight, or no, seed yield increases with Laird lentil, a resistant cultivar. With a susceptible type of lentil, it appeared that one or two applications gave the largest profit/ha. Further investigations are required to determine the most suitable and economic fungicide program to control ascochyta blight of lentil in Saskatchewan.

Erskine, W., Williams, P.C. and Nakkoul, H. 1985. **Genetic and environmental variation in the seed size, protein, yield, and cooking quality of lentils.** *Field Crops Research* 12:153-161. ICARDA, P.O.Box 5466, Aleppo, Syria.

A test of the cooking quality of lentil seed was developed and used on 24 lentil genotypes grown at three rainfed sites in Syria and Lebanon. The repeated sampling and cooking of a standard check gave a coefficient of variation of 4.1% showing the repeatability of the test. The overall average time for cooking was 33 min. The cooking time showed a heritability of $h^2_{bs} = 0.82$. The means of seed yield, protein, and average seed weight were 1223 kg/ha, 27.0%, and 36.9 mg, respectively. Seed yield, protein, and average seed weight gave heritabilities of $h^2_{bs} = 0.50$, 0.71, and 0.98, respectively. There was a negative genetic correlation of $r = -0.937$ between seed yield and protein content, and a positive genetic correlation between cooking time and seed size of $r = 0.919$. Seed size can be used to predict cooking quality. The range in genetic variation in protein was only 3.4%, and increased seed yields can be found without a significant decrease in protein below the level of land races.

Gossen, B.D. 1985. **Ascochyta blight of lentil in Saskatchewan.** *Ph.D. thesis.* University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

The main objectives of the study were (1) to examine the epidemiology of *Ascochyta lentis* Bondartzeva-Monteverde and Vassilievsky, which incites ascochyta blight of lentil (*Lens culinaris* Medik.); (2) to develop recommendations relating to

disease management; and (3) to examine the variability of *A. lentis* relative to *A. fabae* Speg. from faba bean.

The frequency of transmission from seed to seedlings was inversely proportional to soil temperature and directly proportional to the degree of seed discoloration. Stubble-borne inoculum remained highly infective after one winter and resulted in rapid epidemic development early in the growing season. Throughout the season, disease increased explosively under moist and humid conditions. The lentil cultivar Laird was relatively resistant, but Eston and the land race Common Chilean were susceptible to foliar blight. Severe artificially-induced epidemics resulted in seed yield losses of over 40% in susceptible lentils. Seed grade was also reduced due to discoloration of infected seed. Yield loss was related to amount of disease present at mid-season by the equation (derived from regression analysis) % yield loss = $47.\text{Log}_{10}$ (% leaf area affected). Seed discoloration due to blight occurred rapidly under moist conditions in windrows (swaths) left to dry and mature, but the frequency of infected seed was constant after the first seven days.

Analysis of morphological and cultural characters measured on isolates of *A. lentis* and *A. fabae* demonstrated no consistent differences between these two species. Therefore, they should be synonymized under the name *A. fabae*. Studies of the host range and specificity of representative isolates were inconclusive due to the low virulence of many of the isolates.

The use of blight-free seed, a four-year rotation, and resistant cultivars are recommended to minimize blight incidence and intensity.

Hoffman, D.L. 1985. **Species relationships in *Lens* Miller and potential of the wild lentil gene pool for cultivar improvement.** *Ph.D. thesis.* Washington State University, Washington.

Principal component analyses were conducted to characterize phenotypic variation within a *Lens* germplasm collection and to assess species relationships. When several quantitative and qualitative characters were analyzed, *L. orientalis* and *L. nigricans* grouped closest to *L. culinaris*, while *L. ervoides* was the furthest removed. Two *L. nigricans* accessions and one *L. ervoides* accession were graphically removed from their respective groups. When the quantitative characters alone were analyzed, additional *L. nigricans* accessions separated from the remaining *L. nigricans*. This separation of *L. nigricans* was concordant with the recent crossability and cytogenetic studies of Ladizinsky.

Starch gel electrophoresis of isozymes was conducted to ascertain relationships among the *Lens* taxa. The 14 enzymes studied yielded 25 scorable isozyme loci. Isozyme polymorphism within accessions was extremely low, while variation among accessions and among taxa were greater. *L. nigricans* subspecies *nigricans* was found to be the most electrophoretically distinctive taxon, while all the other *Lens* taxa were more similar. The electrophoretic data agreed with G. Ladizinsky's placement of subspecies *orientalis* and *odemensis* with the subspecies *culinaris* within *L. culinaris*, but disagreed with the placement of subspecies *ervoides* with the subspecies *nigricans* within *L. nigricans*.

A breeding study was conducted to evaluate the relative contribution of the wild accessions toward cultivar improvement and to characterize further the primary gene pool of the cultivated lentil. Significant effects due to wild parent were observed for seed yield/plant and harvest index. Crosses of an unadapted cultivar and some *L. orientalis* accessions resulted in progenies with yield performances comparable to the unadapted control. Most *L. orientalis* and ssp. *odemensis* accessions can be utilized by direct hybridization.

Several accessions of wild *Lens* species and two cultivars were studied for differences in vernalization response. All accessions except two, *L. orientalis* and one *L. culinaris* cultivar, responded significantly to vernalization with earlier flowering. This was especially true for a few accessions of *L. nigricans*. A smaller number of accessions responded with longer blooming durations or earlier maturity. No yield differences due to vernalization were detected. The high vernalization requirement in *L. nigricans* may be valuable in developing a winter lentil variety in the temperate areas.

Ladizinsky, G. 1985. **The genetics of hard seed coat in the genus *Lens*.** *Euphytica* 34: 539-543.

Seeds of the cultivated lentil are capable of germinating shortly after maturation. The seed

dormancy of wild lentil species is due to a hard seed coat. In crosses between the cultivated species *L. culinaris* and its wild progenitor *L. orientalis* the hard seed coat of the wild species was controlled by a single recessive gene in homozygous condition. In a cross between the wild species *L. ervoides* and *L. culinaris* the hard seed coat of *L. ervoides* was controlled by a single dominant gene. The significance of the genetics of seed coat hardness in the domestication of lentil is briefly discussed.

Rai, R. 1985. **Studies on associative nitrogen fixation by antibiotic-resistant mutants of *Azospirillum brasilense* with genotypes of lentil (*Lens culinaris*) *Rhizobium* strains in calcareous soil.** *Journal of Agricultural Science* 104:207-215. Rajendra Agricultural University, Dholi Campus (Muzaffarpur), Bihar, 843 121, India.

Nitrosoguanidine-induced mutation frequencies for resistance to streptomycin, spectinomycin, erythromycin, and novomycin were studied in *Azospirillum brasilense*. Lentil inoculated with *A. brasilense* and its mutants and *Rhizobium* strains produced increased nodule dry weight, nitrogenase activity of nodules and roots, and grain yield compared with an uninoculated control.

Rai, R., Nasar, S.K.T., Singh, S.J. and Prasad, V. 1985. **Interactions between *Rhizobium* strains and lentil (*Lens culinaris* Linn.) genotypes under salt stress.** *Journal of Agricultural Science* 104:199-205. Rajendra Agricultural University, Dholi Campus, Dholi (Muzaffarpur), Bihar, 843 121, India.

Three strains of *Rhizobium*, able to fix nitrogen in symbiosis with lentils in saline soil, were screened. Nodulation pattern, N_2-fixation, and grain yield were all influenced by *Rhizobium* strain and lentil genotype. Genotypes DL-443 and Pant L-406 were found to be more salt tolerant than others, and gave the highest grain yield.

Townley-Smith and Slinkard, A.E. 1985. **Annual legume green manure crops in Western Canada.** *Canadian Journal of Plant Science* 65: 241-242. University of Saskatchewan, Crop Development Centre, Saskatoon, Saskatchewan S7N OWO.

Four annual legumes, lentil (*Lens culinaris* 'Eston'), field pea (*Pisum sativum* 'Trapper'), faba bean (*Vicia faba* 'Outlook'), and Tangier flatpea (*Lathyrus tingitanus* 'Tinga'), were compared for their suitability as annual green manure crops grown in the fallow year of a wheat-fallow rotation. As nitrogen fixation usually declines after seed set, crop growth was stopped by cultivation or desiccation before seed filling started. This practice prevented continuing use of available soil moisture, conserving it for the following grain crop. Seasonal total nitrogen fixation, assayed by acetylene reduction, ranged from 8 kg/ha^{-1} for the Tangier flatpea to 38 kg/ha^{-1} for the field pea. However, total aboveground nitrogen at plowdown ranged from 24 to 90 kg/ha^{-1}. The measured nitrogen fixation accounted for 25-32% of the total aboveground nitrogen. In 1983, field pea seed cost approximately \$60/ha^{-1}, while the value of the nitrogen fixed was approximately \$25/ha^{-1}.

Vaillancourt, R., Slinkard, A.E. and Reichert, R.D. 1985. **Inheritance of tannin content in lentil.** *Canadian Journal of Plant Science* 65: 242 University of Saskatchewan, Crop Development Centre and National Research Council, Plant Biotechnology Institute, Saskatoon, Saskatchewan S7N OWO.

The inheritance of tannin (proanthocyanidin) content in lentil (*Lens culinaris* Medik.) was investigated. Crosses were made between zero tannin (P.I. 345635), medium tannin (Redchief, Laird, Eston), and high tannin (P.I. 211602, P.I. 320937) lines. The inheritance of tannin was studied in the F_1 and F_2 from these crosses. Heritability of tannin content was calculated by the component of variance method with four lines at four locations and four replications. Tannins in lentil are concentrated in the seed coat and thus are maternally inherited. All crosses with the zero-tannin line (P.I. 345635), segregated three tannin-containing: one zero-tannin in the F_2, indicating that zero-tannin is controlled by a single recessive gene. Zero-tannin segregants contained no anthocyanin pigmentation in stems, flowers, or seed coats and have seeds that do not darken under storage. Seed coats of the zero-tannin lines are thinner than the tannin-containing lines. Zero-tannin plants are also susceptible to seed rotting and ascochyta blight. The F_2 distribution of tannin content, in crosses between Redchief and P.I. 320937 and P.I. 211602, indicated that tannin content is quantitatively inherited in crosses where neither parent is zero-tannin.

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LENS News Service

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June

II International Legume Conference
St. Louis, Missouri, USA, 23-27 June
Contact: Dr. J.L. Zarucchi, Legume Conference Coordinator, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166, USA

July

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Spokane, Washington, USA, 6-11 July
Contact: Dr. R.H. Lockerman, Chairman, International Food Legume Research Conference, Plant and Soil Science Department, Montana State University, Bozeman, Montana 59717, USA

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